New Hampshire Water Resources Research Center
Annual Technical Report
FY 2017

Introduction

The New Hampshire Water Resources Research Center (NH WRRC), located on the campus of the University of New Hampshire (UNH), is an institute that serves as a focal point for research and information on water issues in the state. The NH WRRC actually predates the Federal program. In the late 1950s Professor Gordon Byers (now retired) began a Water Center at UNH. This Center was incorporated into the Federal program in 1965 as one of the original 14 state institutes established under the Water Resource Research Act of 1964. The NH WRRC is currently directed by Dr. William McDowell with administrative and technical assistance from Associate Director Ms. Michelle Shattuck and Mr. Jody Potter (Water Quality Analysis Lab (WQAL) Manager). The NH WRRC is a standalone organization, in that it is not directly affiliated with any other administrative unit at UNH, and it reports to the Dean of the College of Life Sciences and Agriculture (COLSA). The NH WRRC has no dedicated laboratory or research space, and instead relies on space allocated for the research activities of the WRRC director by COLSA. The NH WRRC does have administrative space on campus, which houses WRRC files and short-term visiting staff and graduate students. The WRRC website (www.wrrc.unh.edu) serves as a focal point for information dissemination and includes NH WRRC publications and results from past research, as well as links to other sites of interest to NH citizens and researchers.

Research Program Introduction

The NH WRRC supported the following research projects in 2017:

1. Water Quality and the Landscape: Long-term monitoring of rapidly developing suburban watersheds (104B)

2. Effects of dissolved organic carbon on methylmercury bioavailability in stream ecosystems (104G)

3. Salt and Streams: Assessing ecological stress in New Hampshire watersheds at community, population, and molecular levels (104B)

4. Ecosystem Indicators for Freshwater Streams (104B)

5. Determining the Effectiveness of the Clean Air Act and Amendments for the Recovery of Surface Waters in the Northeastern U.S. (104S)

6. Hot and Salty: Assessing ecological stress in New Hampshire streams at community, population, and molecular levels (104B)

7. Stormwater and Development: How do New Hampshire's communities address the impacts in the land use planning process? (104B)

The Water Quality Analysis Lab (WQAL) is affiliated with the NH WRRC and facilitates water resources research through technical assistance and sample analysis. The WQAL was established by the Department of Natural Resources in 1996 to meet the needs of various research and teaching projects both on and off the UNH campus. It is currently administered by the NH WRRC and housed in James Hall. The mission of the Water Quality Analysis Laboratory is to provide high-quality, reasonably priced analyses in support of research projects conducted by scientists and students from throughout the University, state, and nation. Past clients have included numerous research groups on the UNH campus, Federal agencies, scientists from other universities, and private firms. Many thousands of analyses are conducted each year.

Water Quality and the Landscape: Long-term monitoring of rapidly developing suburban watersheds

Basic Information

Title:	Water Quality and the Landscape: Long-term monitoring of rapidly developing suburban watersheds
Project Number:	2003NH21B
Start Date:	3/1/2003
End Date:	2/28/2018
Funding Source:	104B
Congressional District:	NH01
Research Category:	Water Quality
Focus Categories:	Non Point Pollution, Surface Water, Nutrients
Descriptors:	
Principal Investigators:	William H. McDowell, Michelle Daley Shattuck

Publications

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Water Quality and the Landscape: Long-term monitoring of rapidly developing suburban watersheds

Statement of Critical Regional or State Water Problem

New Hampshire's surface waters are a very valuable resource, contributing to the state's economic base through recreation (fishing, boating, and swimming), tourism and real estate values, and drinking water supplies. New Hampshire has experienced rapid growth in several counties. From 1990 to 2004 the state grew twice as fast as the rest of New England, with a state-wide average population increase of 17.2% during that period (Society for Protection of NH Forests 2005). New Hampshire's population growth has slowed slightly and from 2000-2010 the state experienced a 6.5% population gain. This was still the largest gain among northeastern states and the fastest-growing areas in New England are concentrated in southern and central NH (Johnson 2012). New Hampshire watersheds rank among the most highly threatened watersheds in the nation because of the high potential for conversion of private forests to residential development. In fact, three of the four most threatened watersheds in the US which could experience the largest change in water quality as a result of increased residential development in private forests occur at least partially in New Hampshire (Stein et al. 2009).

The long-term impacts of this rapid population growth and the associated changes in land use on New Hampshire's surface waters are uncertain. Of particular concern are the impacts of non-point sources of pollution such as septic systems, urban runoff, stormwater, application of road salt and fertilizers, deforestation, and wetland conversion. Long-term datasets that include seasonal and year-to-year variability in precipitation, weather patterns and other factors are needed to adequately document the cumulative effects of land use change and quantify the effectiveness of watershed management programs. No other agency or research program (e.g. NH Department of Environmental Services (NH DES), US Geological Survey (USGS) or Environmental Protection Agency (EPA)) has implemented such a long-term program.

Statement of Results or Benefits

This project provides detailed, high-quality, long-term datasets which allow for a better understanding of the impacts of land use change and development on surface water quality. These surface water datasets could support the development, testing and refinement of predictive models, accurately assess the impacts of watershed management practices on drinking water supplies, assess efforts to reduce surface water quality impairments, and be potential early warning signs of dramatic changes to surface water quality in the region resulting from rapid development. Long-term datasets from this project will be essential to adaptive management strategies that strive to reduce non-point sources of nitrogen pollution in New Hampshire's Great Bay watershed where several estuarine waters are currently impaired by elevated nitrogen and in violation of the Federal Clean Water Act. A list of selected recent presentations, publications and press releases that utilize long-term datasets supported by NH WRRC funding for this project is included at the end of this report.

Objectives of the Project

This project allows for the continued collection of long-term water quality data in New Hampshire. It will use University of New Hampshire (UNH) staff, students and volunteers from local communities to collect samples from the Lamprey and Oyster River watersheds located in southeast NH and the Ossipee River watershed in central NH. All three watersheds are located in counties experiencing high population growth rates (Figure 1). Both the Lamprey and Ossipee watersheds are predicted to more than double in population from 1998 to 2020 (Sundquist and Stevens 1999). Surface water sites within each of the 3 watersheds and details on long-term datasets collected are described below. Together these 3 watersheds capture a broad range of urban, rural and agricultural land uses as well as a range of forests and wetland cover types.

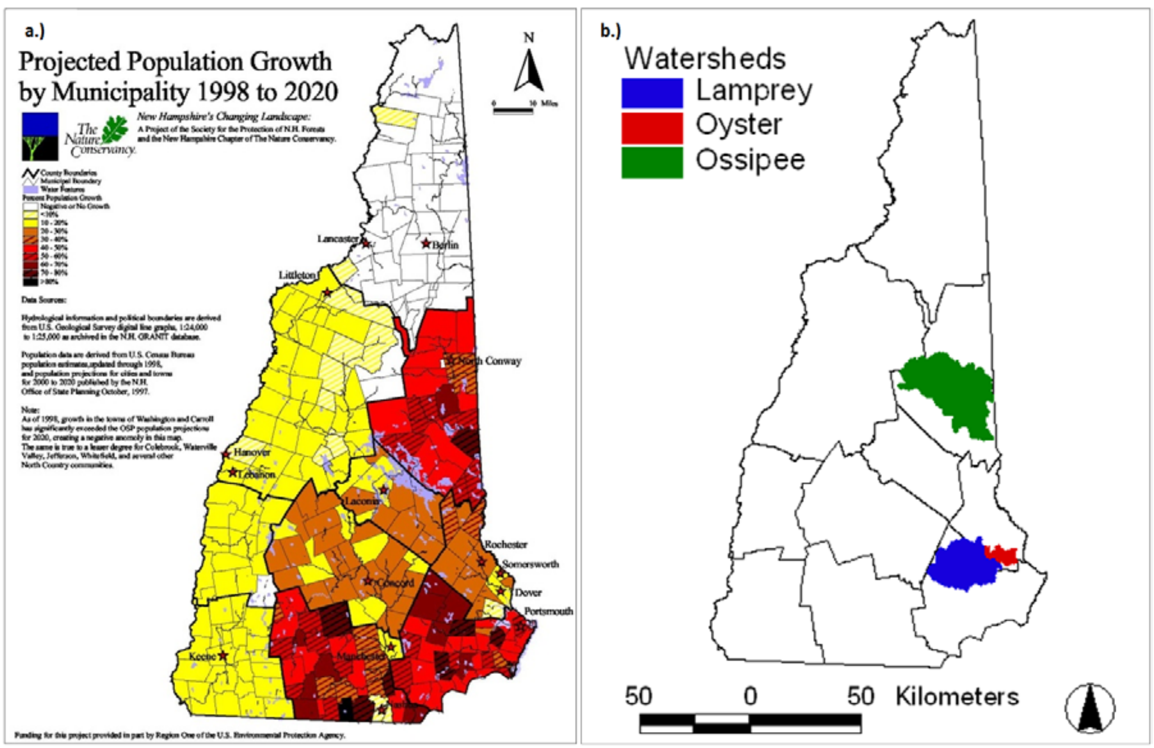

Figure 1. Projected population growth in New Hampshire (Figure from Sundquist and Stevens 1999; A) and study watersheds experiencing high population growth (B).

Methods, Procedures and Facilities

Lamprey River Hydrologic Observatory

The Lamprey River watershed (479 km^2) is a rural watershed located in southeastern NH and is under large development pressure as the greater area experiences the highest population growth in the state. The Lamprey River Hydrologic Observatory (LRHO) is a name given to the entire Lamprey River basin as it serves as a platform to study the hydrology and biogeochemistry of a suburban basin and is used by the UNH community as a focal point for student and faculty research, teaching and outreach. Our goal for the long-term Lamprey water quality monitoring program is to document changes in water quality as the Lamprey watershed becomes increasingly more developed and to understand the controls on N transformations and losses.

The Lamprey River has been sampled weekly and during major runoff events since September 1999 at site LMP73 which is co-located with the Lamprey River USGS gauging station (01073500) in Durham, NH. Two additional sites were added to the long-term Lamprey River monitoring program in January 2004. One site (NOR27) was located on the North River, the Lamprey River's largest tributary, less than 1 km downstream from the USGS gauging station (01073460) in Epping, NH. The other site (Wednesday Hill Brook; site WHB01) drains a small suburban area in Lee, NH where residents rely solely on private wells and private septic systems for water supply and waste disposal. A stream gauge at WHB01 is operated by UNH staff and/or students. Sites NOR27 and WHB01 were sampled on a weekly basis through 2010 and in January 2011, the North River sampling frequency (site NOR27) was reduced to monthly because accurate measures of river discharge were no longer possible. Site WHB01 along with LMP73 remain at a weekly and major storm event sampling frequency. Several other sites have been sampled for multiple years on a less frequent basis to assess the spatial variability of water quality in sub-basins with various land uses and development intensities. In the past year, 14 additional sites were sampled on a monthly basis. All LRHO stream water samples are collected by UNH staff and/or students.

Oyster River watershed

The Oyster River watershed (80 km^2) is a small watershed in southeast NH where land use ranges from rural to urban. Two urban sub-basins, College Brook (CB) and Pettee Brook (PB), were selected for long-term sampling in January 2004. Both sub-basins are dominated by the University of New Hampshire (UNH) and receive a variety of non-point pollution from several different land uses. Three sites (CB00.5, CB01.5 and CB03.0) are sampled along College brook which drains the center of campus and one site (PB02.0) is located on Pettee Brook which drains the northern section of campus. Both sub-basins drain areas with high amounts of impervious surface and College Brook also drains the UNH dairy farm and athletic fields. Historic water quality data for these two sites are available from 1991. UNH staff and/or students currently sample these sites on a monthly basis.

Ossipee River watershed

The entire Ossipee River watershed (952 km^2) is classified as rural due to its low but increasing population. Seven sites in the watershed were selected for long-term monitoring in May of 2004. These sites are monitored monthly by volunteers and staff of the Green Mountain Conservation Group (GMCG) and were chosen to capture the areas of concentrated growth and monitor the major inputs and outputs from Ossipee Lake. Additional sites are selected by GMCG for volunteer monitoring during non-winter months (May to November). WRRC staff assist GMCG in site selection and data interpretation. In 2006, the GMCG worked with the Department of Environmental Services to establish a Volunteer Biological Assessment Program (VBAP) for the Ossipee Watershed. Numerous volunteers, including students from five local schools, assist with invertebrate sampling at a total of eleven sites.

Water Quality Analysis

Field parameters (pH, conductivity, dissolved oxygen (DO) and temperature) are measured at all sites. Water samples are filtered in the field using pre-combusted glass fiber filters (0.7 µm pore size), and frozen until analysis of dissolved constituents. Samples collected at all LRHO, CB, PB and the 7 long-term GMCG sites are analyzed for dissolved organic carbon (DOC), total dissolved nitrogen (TDN), nitrate (NO_3-N), ammonium (NH_4-N), dissolved organic nitrogen (DON), orthophosphate (PO_4-P), chloride (Cl^-), sulfate (SO_4-S), sodium (Na^+), potassium (K^+), magnesium (Mg^{+2}), calcium (Ca^{+2}), and silica (SiO_2). Water chemistry is also analyzed on a sub-set of the GMCG seasonal sites and turbidity is measured in the field at all GMCG sites. Samples collected since October 2002 from LMP73 are also analyzed for total suspended sediment (TSS), particulate carbon (PC), particulate nitrogen (PN) and dissolved inorganic carbon (DIC). All samples are analyzed in the Water Quality Analysis Laboratory (WQAL) of the NH WRRC on the campus of UNH, Durham, NH. Methods for analyses include ion chromatography (Cl^-, NO_3^-, SO_4^{-2} and Na^+, K^+, Mg^{+2}, Ca^{+2}), discrete colorimetric analysis (NH_4, PO_4, NO_3/NO_2), and High Temperature Oxidation (DOC, TDN). All methods are widely accepted techniques for analysis of each analyte.

The WQAL was established by the Department of Natural Resources in 1996 to meet the needs of various research and teaching projects both on and off the UNH campus. It is currently administered by the NH Water Resources Research Center and housed in James Hall. Dr. William McDowell is the Laboratory Director and Mr. Jody Potter is the Laboratory Manager. Together, they have over 45 years of experience in water quality analysis, and have numerous publications in the fields of water quality, biogeochemistry, and aquatic ecology.

Principal Findings and Significance

Lamprey River Hydrologic Observatory

Analysis of samples collected in 2017 from the LRHO is approximately 50% complete. Results of stream chemistry to date show a significant increase in annual nitrate concentrations at LMP73 (Figure 2) over the entire study period (2000-2016), but not at WHB01. We have shown previously that stream water nitrate is related to watershed population density (Daley 2002) and since suburbanization continues to occur throughout the greater Lamprey River watershed, population growth is likely responsible for the increase in stream water nitrate over the study period. The watershed population density increased from 53 to 60 people/km^2 or by 12% from 2000 to 2010 (2000 and 2010 Census). The highest levels of nitrate at LMP73 occurred in 2014. We are uncertain if nitrate levels in LMP73 will remain relatively constant, increase or decrease with changing climate, land use and management in the watershed. Wednesday Hill Brook watershed is near its development capacity, unless the Town of Lee, NH changes its zoning regulations, and the lack of increase in WHB01 nitrate may be due to the limited population growth in this watershed, that this watershed has reached nitrogen saturation or that the current time period of data collection is not reflective of long-term trends. Changes in Lamprey River nitrogen, especially nitrate, can have significant impacts for the downstream receiving water body, the Great Bay estuarine system which is impaired by elevated nitrogen and is currently in violation of the Federal Clean Water Act. Tidal tributaries to the bay are experiencing dangerously low dissolved oxygen levels and the bay is experiencing a significant loss of eelgrass which provides important habitat for aquatic life. The Lamprey River is the largest tributary to Great Bay, and thus the long-term data provided by the NH WRRC from the LRHO are of considerable interest for watershed management.

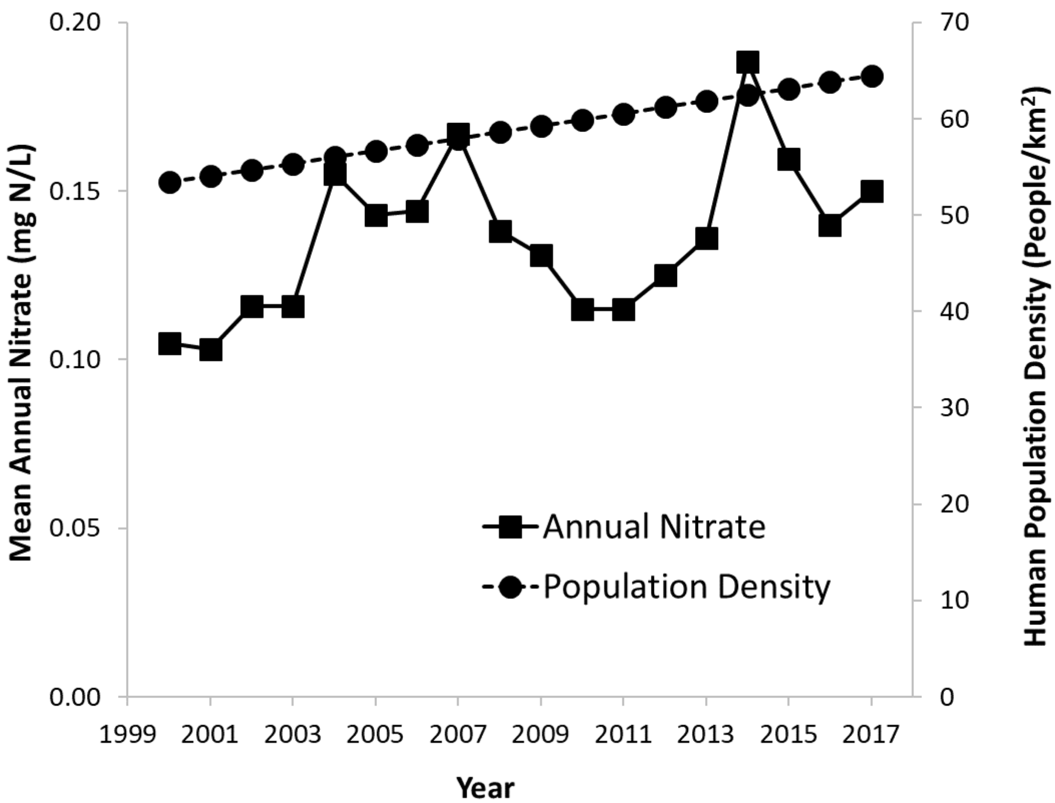

Figure 2. Annual mean nitrate concentration and estimated annual human population density (2000 and 2010 Census) from 2000-2017 in the Lamprey River basin. Note that nitrate analysis for 2017 is approximately 50% complete.

When we combine our specific conductance data (2003 – 2017) with data collected by the USGS (1978 - 1999), we see a long-term increase in specific conductance in the Lamprey River (Figure 3). Sodium and chloride concentrations are directly related to specific conductance ($r^2 = 0.95$, $p<0.01$ for Na^+; $r^2 = 0.93$, $p < 0.01$ for Cl^-) and we conclude that this increase in specific conductance indicates a corresponding increase in Lamprey River NaCl. Since Na^+ and Cl^- are strongly correlated with impervious surfaces in southeast NH (Daley et al. 2009) and road pavement among southeastern and central NH basins, we conclude that the associated road salt application to these surfaces is responsible for this long-term increase in stream water NaCl. Like nitrate, we are uncertain if NaCl levels in LMP73 will remain relatively constant, increase or decrease with changing climate, land use and management in the watershed.

Thus, continued monitoring is necessary to better understand how the interaction between human activities and climate variability affects water quality.

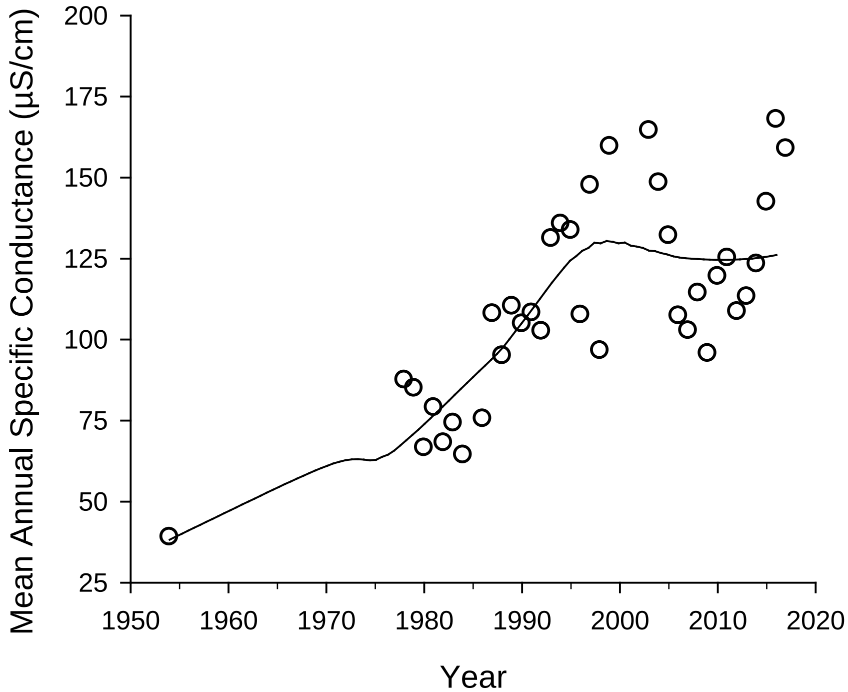

Figure 3. Mean annual specific conductance in the Lamprey River at LMP73 (co-located with the USGS gauging station in Durham, NH. (modified from Daley et al. 2009).

Oyster River watershed

Laboratory analysis of the monthly CB and PB samples collected in 2017 is approximately 50% complete. Recent data show that DO is lowest at the CB upstream station (CB00.5) where it does drop below 5 mg/L (level that is necessary to support in-stream biota) during the summer months. The downstream stations do not drop below 5 mg/L and this difference is due to the hydrologic and biogeochemical properties of the upstream sampling location which has slow stream flow, high dissolved organic matter content and resembles a wetland. DO increases downstream as flow becomes faster and the stream is re-aerated.

Data from 2000 until now indicate that the steam is strongly impacted by road salt application at its origin, which is essentially a road-side ditch along the state highway leading to a wetland area, and by road salt applied by UNH and the town of Durham which drains to the middle and lower reaches of the brook (Figure 4). Average sodium and chloride concentrations, as well as specific conductance, appear to have remained reasonably constant since 2001, but are much higher than in 1991 (Daley et al. 2009). Concentrations are highest at the upstream stations and tend to decline downstream as the stream flows through the campus athletic fields and then increase as the stream passes through the heart of campus and downtown Durham. Concentrations are also highest during years of low flow. Data from this project have been used to list College Brook as impaired for excess chloride.

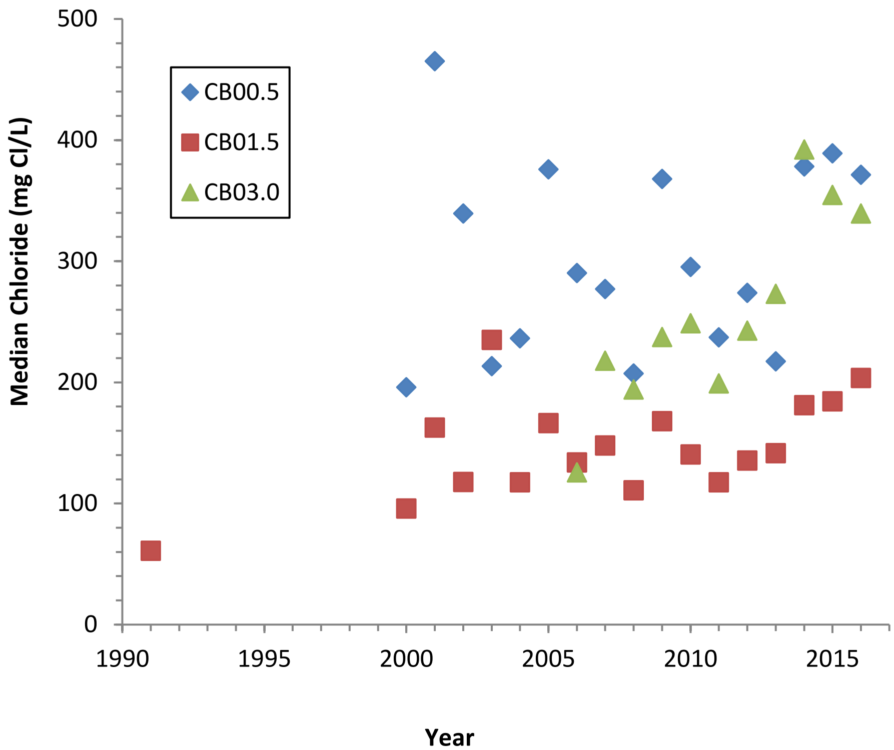

Figure 4. Median annual chloride in College Brook from the headwaters (CB00.5) to the mouth (CB03.0).

College Brook and Pettee Brook have noticeably higher nitrogen concentrations than many other local streams draining less developed or undeveloped watersheds. As College Brook flows from upstream to downstream where it becomes more aerated, ammonium decreases and nitrate increases indicating that nitrification is occurring in the stream channel. However, an increase in dissolved inorganic nitrogen (DIN) and total dissolved nitrogen (Figure 6) from the headwaters to the mouth indicates that there are additional sources of nitrogen entering the stream as it flows though UNH and Durham. This is possibly from fertilization of the athletic fields, storm water runoff or exfiltration from sewage lines. There is no statistically significant change in nitrate, DIN or TDN concentrations from 2000 to 2017 at the station with the longest record (CB01.5).

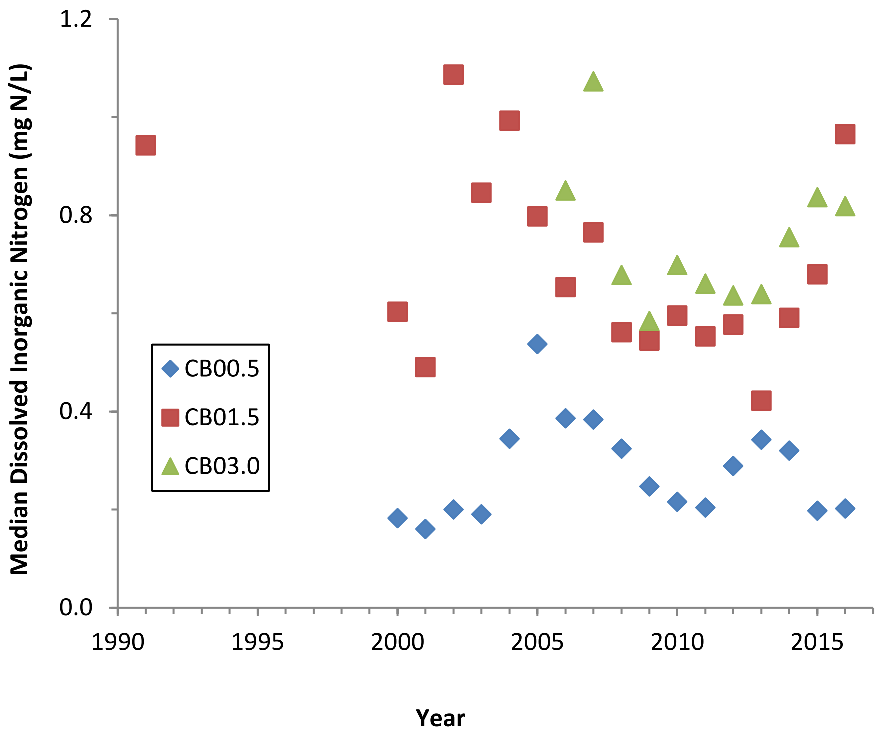

Figure 5. Median annual dissolved inorganic nitrogen (DIN) in College Brook from the headwaters (CB00.5) to the mouth (CB03.0).

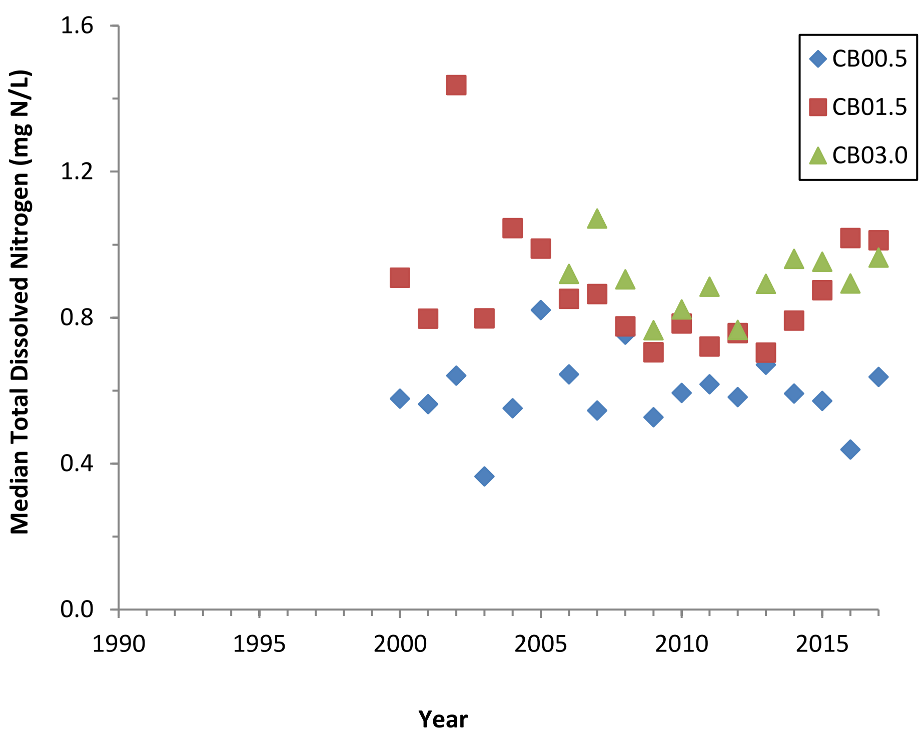

Figure 6. Median annual total dissolved nitrogen (TDN) in College Brook from the headwaters (CB00.5) to the mouth (CB03.0).

Ossipee Watershed

Collaboration with the Green Mountain Conservation Group (GMCG) and their sampling of the Ossipee River watershed provides much benefit to the NH WRRC and the long-term monitoring of rapidly developing suburban watersheds. Volunteers sampled streams within the watershed every 2 weeks from April through October, and monthly winter sampling was conducted by volunteers and GMCG staff at 9 sites. Over 100 samples were collected for analysis in the WQAL and additional field data were collected at over 40 sites throughout 6 towns using the help of many volunteers. Many presentations were made to planning boards, conservation commissions and other local government groups. The impact of road salting in this central NH watershed is similar to what we see in coastal NH (Daley et al. 2009). Data have been used to heighten awareness of the impacts of excessive road salting and snow dumping in local streams. Communication with local road agents has led to the remediation in one development where road salting was an issue. Samples collected and data generated from this funding have shown an improvement in water chemistry following reduced salting and snow dumping. Data have also been useful in promoting low impact development techniques and best management practices where new development has been proposed in proximity to lakes, rivers and streams within the watershed.

Number of students supported

Three PhD students (Lauren Koenig, Bianca Rodriguez, Rich Brereton), two MS students (Alison Herreid and Brian Saccardi) and 8 undergraduate hourly employees from the Department of Natural Resources & the Environment (John Ciaburri, Justin Sherman, Liam Waldron, Christina Lyons, Kyle Seawards, Light Mcharo, Danielle Chancey and Brooke Gauthier). Three post-doctoral students were also supported by this project (Adam Wymore, Ashley Coble and Qingtao Zhou).

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Daley, M.L. May 2002. Export of Dissolved Organic Carbon, Dissolved Organic Nitrogen and Nitrate from the Lamprey River Watershed, New Hampshire: Examining Relationships with Watershed Characteristics. Master's Thesis, University of New Hampshire, Durham, NH.

Daley, M.L., J.D. Potter and W.H. McDowell. 2009. Salinization of urbanizing New Hampshire streams and groundwater: impacts of road salt and hydrologic variability. Journal of the North American Benthological Society 28(4):929–940.

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Medalie, L. 2013. Concentrations of chloride and sodium in groundwater in New Hampshire from 1960 through 2011. USGS Fact Sheet 2013-3011. (Available at: http://pubs.usgs.gov/fs/2013/3011/).

Society for Protection of NH Forests. 2005. New Hampshire's Changing landscape. Population growth and land use changes: What they mean for the Granite State. (Available at: http://www.spnhf.org/research/papers/nhcl2005es.pdf).

Stein, S.M., McRoberts, R.E., Mahal, L.G., Carr, M.A., Alig, R.J., Comas, S.J., Theobald, D.M. and Cundiff, A. 2009. Private forests, public benefits: increased housing density and other pressures on private forest contributions. Gen. Tech. Rep. PNW-GTR-795. Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station. 74 p.

Sundquist D and Stevens M. 1999. New Hampshire's changing landscape. Population growth, land use conservation, and resource fragmentation in the Granite State. Society for the Protection of New Hampshire Forests, Concord, New Hampshire. 110 pp.

Notable awards and achievements

McDowell, W.H. 2017. Received the 2017 Distinguished Professor Award. The purpose of this award is to identify and honor longstanding members of the faculty. This singular university-wide award will be given each year to the faculty member whose overall record of excellent teaching, caring about students, devotion to the university community and substantial record of scholarly achievement exemplifies what we would call a 'distinguished career'.

Rodriguez-Cardona, B. (PhD student) was awarded a 2017 Chateaubriand Fellowship. The Chateaubriand Fellowship is a grant offered by the Embassy of France in the United States. It supports outstanding Ph.D. students from American universities who wish to conduct research in France for a period ranging from 4 to 9 months. Chateaubriand fellows are selected through a merit-based competition, through a collaborative process involving expert evaluators in both countries. Bianca has travelled the globe, studying carbon and nitrogen coupling in streams across biomes. She traveled to Paris, France for this fellowship.

Information transfer activities that utilize long-term datasets supported by NH WRRC and matching funds

Publications

Bucci, J. P., M. D. Shattuck, S. A. Aytur, R. Carey and W. H. McDowell (2017). A case study characterizing animal fecal sources in surface water using a mitochondrial DNA marker. Environmental Monitoring and Assessment 189(8).

Contosta, A. R., A. Adolph, D. Burchsted, E. Burakowski, M. Green, D. Guerra, M. Albert, J. Dibb, M. Martin, W. H. McDowell, M. Routhier, C. Wake, R. Whitaker and W. Wollheim (2017). A longer vernal window: the role of winter coldness and snowpack in driving spring transitions and lags. Global Change Biology 23(4): 1610-1625.

Hunt, C. W., L. Snyder, J. E. Salisbury, D. Vandemark and W. H. McDowell (2017). SIPCO2: A simple, inexpensive surface water pCO(2) sensor. Limnology and Oceanography-Methods 15(3): 291-301.

Koenig, L.E., Shattuck, M.D., Snyder, L.E., Potter, J.D. and McDowell, W.H. 2017. Deconstructing the effects of flow on DOC, nitrate, and major ion interactions using a high-frequency aquatic sensor network. Water Resources Research. 53: 10,655–10,673. DOI: 10.1002/2017WR020739.

Snyder, L.E., Potter, J.D. and McDowell, W.H. 2018. An Evaluation of Nitrate, fDOM, and Turbidity Sensors in New Hampshire Streams. Water Resources Research. Special issue "Continuous nutrient sensing in research and management: applications and lessons learned across aquatic environments and watersheds". DOI: 10.1002/2017WR020678

Wymore, AS, J Potter, L Snyder, B Rodríguez-Cardona, and WH McDowell. 2018. Using in-situ optical sensors to understand the biogeochemistry of dissolved organic matter across a stream network. Water Resources Research. DOI: 10.1002/2017WR022168.

Conference Proceedings & Abstracts:

Contosta, A., Burchsted, D., Burakowski, E., Green, M., Guerra, D., Albert, M., Dibb, J., Martin, M., McDowell, W.H., Routhier, M., Wake, C., Whitaker, R. and Wollheim, W. 2017. A longer vernal window: How winter coldness and snowpack influence spring transitions and lags. Northeast Ecosystem Research Cooperative Conference. March 28-29, 2017, Saratoga Springs, New York, USA.

Koenig, L.E., L.E. Snyder, A.P. Appling, C. Hunt, J.D. Potter, W.H. McDowell. 2017. Annual patterns in aquatic metabolism and CO2 emissions from New Hampshire streams (Oral). June 4-9, 2017 Society for Freshwater Science meeting, Raleigh, NC.

McDowell, W.H., J. Potter. 2017. Tradeoffs in Greenhouse Gas Fluxes from Aquatic Ecosystems Along a Rural to Urban Gradient are Driven by N Loading. 2017 Fall Meeting, AGU, New Orleans, LA, 11-15 Dec.

Shattuck, M.D. and McDowell, W.H. 2017. U.S. Geological Survey - New England Water Science Center Science Symposium: Coastal Nitrogen Research. April, 18, 2017. Worcester, MA.

Shattuck, M.D., Koenig, L. Potter, J.D., Snyder, L.E. and McDowell, W.H. 2017. Regional coherence in solute interactions during stormflow in a statewide aquatic sensor network. NH Water & Watershed Conference. March, 24, 2017. Plymouth, NH.

Wymore, A., S. Kaushal, W.H. McDowell, P. Kortelainen, E. Bernhardt, P. Johnes, W. Dodds, S. Johnson, J. Brookshire, R. Spencer, B.Rodriguez-Cardona, A. Helton, R. Barnes, A. Algerich, S. Haq, P. Sullivan, C. Lopez-Lloreda, A. Coble, M.D. Shattuck. 2017. Carbon and nitrogen stoichiometry across stream ecosystems. 2017 Fall Meeting, AGU, New Orleans, LA, 11-15 Dec.

Presentations/Information Transfer

Koenig, L. 2017. For the 5th consecutive year, Koenig served as the instructor for the STEM mini-course offered August 21st-25th, 2017 through the CONNECT program at UNH (http://www.unh.edu/connect/). The objective of the course is to provide an opportunity for incoming freshmen that come from groups with historically low retention in STEM majors (e.g. low-income, multicultural, first-generation college students) to build community, discover college resources, and bolster skills that are needed to succeed in their academic programs (e.g. writing of lab/research reports, basic math and statistics for analyzing scientific data). There were 13 students in the class, but the broader CONNECT program served approximately 80 students in 2017.

McDowell, W.H. 2017. Taking river biogeochemistry into the fourth dimension. Presentation to NSF EPSCoR program officers. 20 April, 2017.

McDowell, W.H. 2017. The NH Agricultural Experiment Station at the University of New Hampshire welcomed Dr. Catalino Blanche, national program leader in the Division of Environmental Systems with the U.S. Department of Agriculture. Dr. Blanche provides national leadership for forestry and research programs related to the production, protection, and utilization of forest resources, including for the

McIntire-Stennis Cooperative Forestry Program. Dr. McDowell discussed with Dr. Blanche the nitrogen issues in Great Bay and the role of nitrogen cycling in suburbanizing forested watersheds that drain to the bay. Dr. McDowell also discussed the NH EPSCoR Ecosystems & Society project's investment in scientific instrumentation to build a sensor network and showed Dr. Blanche the aquatic sensors installed in the Lamprey River (the largest tributary to Great Bay) at Wiswall Dam in Durham, NH. April 26, 2017.

McDowell, W.H. and Shattuck, M. D. "Nonpoint Nitrogen Sources and Transport in New Hampshire's Great Bay Watershed". Presented 31 October 2017, at the workshop entitled "Nitrogen, Septic Systems, Great Bay and Why it Matters", Great Bay National Estuarine Research Reserve, Greenland, NH.

Shattuck, M.D. and McDowell, W.H. 2017 Nonpoint nitrogen sources and transport in the Great Bay watershed. Unitarian Universalist Church. April 30, 2017. Durham, NH.

Determining the Effectiveness of the Clean Air Act and Amendments for the Recovery of Surface Waters in the Northeastern U.S.

Basic Information

Title:	Determining the Effectiveness of the Clean Air Act and Amendments for the Recovery of Surface Waters in the Northeastern U.S.
Project Number:	2014NH192S
USGS Grant Number:	
Sponsoring Agency:	EPA
Start Date:	7/26/2014
End Date:	1/31/2018
Funding Source:	104S
Congressional District:	
Research Category:	Not Applicable
Focus Categories:	None, None, None
Descriptors:	None
Principal Investigators:	

Publications

1. Brown, R., J. Saros S. Nelson. 2016. Using paleolimnological evidence to assess the consequences of increased dissolved organic carbon in recent decades in lakes of the Northeastern US. J Paleolimnol, in review.
2. Boeff, K.A., K.E. Strock, J.E. Saros. 2016. Evaluating planktonic diatom response to climate change across three lakes with differing morphometry. J Paleolimnol. DOI 10.1007/s10933-016-9889-z.
3. Strock, K.E., Saros, J.E., Nelson, S.J., S.D. Birkel, J.S. Kahl, W.H. McDowell. 2016. Extreme weather years drive episodic changes in lake chemistry: implications for recovery from sulfate deposition and long-term trends in dissolved organic carbon. Biogeochemistry, 127(2-3), 353-365.
4. Brown, R., J. Saros S. Nelson. 2016. Using paleolimnological evidence to assess the consequences of increased dissolved organic carbon in recent decades in lakes of the Northeastern US. J Paleolimnol, in review.
5. Boeff, K.A., K.E. Strock, J.E. Saros. 2016. Evaluating planktonic diatom response to climate change across three lakes with differing morphometry. J Paleolimnol. DOI 10.1007/s10933-016-9889-z.
6. Strock, K.E., Saros, J.E., Nelson, S.J., S.D. Birkel, J.S. Kahl, W.H. McDowell. 2016. Extreme weather years drive episodic changes in lake chemistry: implications for recovery from sulfate deposition and long-term trends in dissolved organic carbon. Biogeochemistry, 127(2-3), 353-365.
7. Soranno, P.A., and 79 others, including S.J. Nelson, W.H. McDowell, 2017. LAGOS-NE: A multi-scaled geospatial and temporal database of lake ecological context and water quality for thousands of U.S. lakes. Submitted, GigaScience (GIGA-D-17-00112).
8. Gavin, A.L., S.J. Nelson, A.J. Klemmer, I.J. Fernandez, K.E. Strock, W.H. McDowell, 2017. Climate linkages to increases of dissolved organic carbon in acid-sensitive high elevation lakes. Submitted, Water Resources Research (2017WR020963).

9. Brown, R.E., S.J. Nelson, J.E. Saros, 2016. Paleolimnological evidence of the consequences of recent increased dissolved organic carbon (DOC) in lakes of the northeastern USA. Journal of Paleolimnology, 57(1), 19-35.
10. Hunt , C. W., Snyder, L., Salisbury, J.E., Vandemark, D., McDowell, W.H. 2017. SIPCO2: A simple, inexpensive surface water pCO2 sensor. Limnology and Oceanography Methods. doi: 10.1002/lom3.10157.
11. Boeff, K.A., K.E. Strock, J.E. Saros. 2016. Evaluating planktonic diatom response to climate change across three lakes with differing morphometry. J Paleolimnol. 56(1), 33-47.
12. Strock, K.E., Saros, J.E., Nelson, S.J., S.D. Birkel, J.S. Kahl, W.H. McDowell. 2016. Extreme weather years drive episodic changes in lake chemistry: implications for recovery from sulfate deposition and long-term trends in dissolved organic carbon. Biogeochemistry, 127(2-3), 353-365.
13. Brown, R., J. Saros S. Nelson. 2016. Using paleolimnological evidence to assess the consequences of increased dissolved organic carbon in recent decades in lakes of the Northeastern US. J Paleolimnol, in review.
14. Boeff, K.A., K.E. Strock, J.E. Saros. 2016. Evaluating planktonic diatom response to climate change across three lakes with differing morphometry. J Paleolimnol. DOI 10.1007/s10933-016-9889-z.
15. Strock, K.E., Saros, J.E., Nelson, S.J., S.D. Birkel, J.S. Kahl, W.H. McDowell. 2016. Extreme weather years drive episodic changes in lake chemistry: implications for recovery from sulfate deposition and long-term trends in dissolved organic carbon. Biogeochemistry, 127(2-3), 353-365.
16. Soranno, P.A., and 79 others, including S.J. Nelson, W.H. McDowell, 2017. LAGOS-NE: A multi-scaled geospatial and temporal database of lake ecological context and water quality for thousands of U.S. lakes. Submitted, GigaScience (GIGA-D-17-00112).
17. Gavin, A.L., S.J. Nelson, A.J. Klemmer, I.J. Fernandez, K.E. Strock, W.H. McDowell, 2017. Climate linkages to increases of dissolved organic carbon in acid-sensitive high elevation lakes. Submitted, Water Resources Research (2017WR020963).
18. Brown, R.E., S.J. Nelson, J.E. Saros, 2016. Paleolimnological evidence of the consequences of recent increased dissolved organic carbon (DOC) in lakes of the northeastern USA. Journal of Paleolimnology, 57(1), 19-35.
19. Hunt , C. W., Snyder, L., Salisbury, J.E., Vandemark, D., McDowell, W.H. 2017. SIPCO2: A simple, inexpensive surface water pCO2 sensor. Limnology and Oceanography Methods. doi: 10.1002/lom3.10157.
20. Boeff, K.A., K.E. Strock, J.E. Saros. 2016. Evaluating planktonic diatom response to climate change across three lakes with differing morphometry. J Paleolimnol. 56(1), 33-47.
21. Strock, K.E., Saros, J.E., Nelson, S.J., S.D. Birkel, J.S. Kahl, W.H. McDowell. 2016. Extreme weather years drive episodic changes in lake chemistry: implications for recovery from sulfate deposition and long-term trends in dissolved organic carbon. Biogeochemistry, 127(2-3), 353-365.
22. Gavin, A.L., S.J. Nelson, A.J. Klemmer, I.J. Fernandez, K.E. Strock, W.H. McDowell, 2017. Climate linkages to increases of dissolved organic carbon in acid-sensitive high elevation lakes. Accepted; available online. Water Resources Research (2017WR020963).
23. Soranno, P.A., and 79 others, including S.J. Nelson, W.H. McDowell, C. Funk, J. Lynch, 2017. LAGOS-NE: A multi-scaled geospatial and temporal database of lake ecological context and water quality for thousands of U.S. lakes. GigaScience, gix101, https://doi.org/10.1093/gigascience/gix101
24. Nelson, S.J., Capone, S.B., Dukett, J.E., Houck, N. 2017. Lake Site Assessments: US EPA TIME-Adirondack Lakes. Report to US EPA and NYSERDA.
25. Gavin, A.L. 2018 (expected). Physical & chemical response of small, north temperate lakes to recovery from acidification & climate change M.S. Thesis, University of Maine, Orono, Maine.
26. Patel, K.F. 2018 (expected). Nitrogen cycling during a period of environmental change. Ph.D. Dissertation, University of Maine, Orono, Maine.

Final five-year Report to

USGS WRD WRRI, Reston, VA
US EPA, CAMD, Washington DC
and US EPA, ORD, Corvallis OR

May 2018

Determining the effectiveness of the Clean Air Act and Amendments on the recovery of surface waters in the northeastern US

IAG 06HQGR0143

Principal Investigators: *William H. McDowell[1], Sarah J. Nelson[2], J. Steve Kahl[1], J. Saros[2]*
[1]Univ. of New Hampshire, [2]Univ. of Maine

Overview of activities during 2013-2018. A schematic summary of progress on the project plan is provided below (Table 1) and discussed on the following pages. We have concluded the final year of five for the most current project agreement, which supports the continuing needs of EPA to assess the effectiveness of the Clean Air Act Amendments of 1990 (CAAA). Field work and data assessment continue on schedule. Project coordination as well as most analytical chemistry is conducted by the University of New Hampshire. Field sampling, some analytical chemistry, data quality assurance, and data reporting are conducted by the University of Maine. Also, one graduate student at the University of Maine was partly funded through this project and also supported by UMaine funds that leveraged this project. Since 2013, there have been seven publications by five graduate students who were partly supported or used LTM data. One research faculty at the University of Maine was partly supported during this project year to develop R code for data QA and analysis, and begin to transition data management to a new secure server. This project year, we contributed data to the LAGOS-NE database and were co-authors on a GigaScience paper that described this "multi-scaled geospatial and temporal database of lake ecological context and water quality for thousands of U.S. lakes" (Soranno et al. 2017). Over the course of this five-year period, we have contributed data to multiple synthesis efforts, including Crossman et al. (2016), Phelan et al. (2016), Lawrence et al. (2015), and current requests by Creed et al. Additionally, this project continues to fund a portion of the base program of stream chemistry monitoring at Bear Brook Watershed in Maine (BBWM), for the reference watershed, East Bear. During this project period, BBWM completed a three-year NSF DEB grant that is evaluating nitrogen dynamics in both watersheds using ^{15}N tracer studies. The base funding through this IAG project created continuity that was key in securing the follow-on NSF award (via RAPID, beginning 01/01/2017, which is assessing the response to discontinuation of experimental acidification). 2016 was the final year for annual TIME lake sampling, both in the New England and Adirondack regions. Co-PI Nelson worked with Adirondack Lakes Survey Corporation staff to complete a compendium summarizing status and research regarding the Adirondack TIME lakes (Nelson et al. 2017). On May 16–17 2017, UMaine hosted the Annual LTM Project Partners meeting at Schoodic Institute in Acadia National Park.

Table 1. 2012-2018 Project plan progress to date.

	2012				2013				2014				2015				2016				2017				2018
Project Activity	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1
project period																									
funding received																									
RLTM drainage																									
RLTM seepage																									
original LTM																									
HELM subset																									
BBWM - EB																									
TIME New England																									
TIME Adirondacks																									
sample analyses																									
Data submission																									
annual report																									

= project plan   = in progress   = completed   = cancelled (weather)

Project background

Objectives. This research is part of EPA CAMD programs that are verifying the effectiveness of emission controls at reducing acidification of surface waters. Our approach is to collect long-term high-quality data that characterize the trends and patterns of response in low ionic-strength surface waters. We have specifically targeted waters that have been classified as being sensitive to acidic deposition and will represent lakes across the Northeast in varying landscape settings. The goals and methods are hierarchical, ranging from intensive site-specific investigations to regional assessment of sites that have been chosen to provide a statistically rigorous sample of regional surface waters. The objectives are to:

1) document the changes and patterns in aquatic chemistry for defined sub-populations and sites that are known to be susceptible to acidification or recovery;
2) evaluate the extent to which changes in surface waters, if any, can be linked to changes in deposition that are driven by regulatory actions;
3) characterize the effectiveness of the CAAA in meeting goals of reducing acidification of surface waters and improving biologically-relevant chemistry in the northeastern US;
4) provide information for assessment of the need for future reductions in atmospheric deposition based on the long-term trajectories of the systems under study; and
5) assess the extent to which increased variability in precipitation events will play a role in the long-term sustainability of CAAA success in these sensitive surface waters. This is leveraged through other funded research.

Approach. The schedule of tasks ranges from weekly to annual, continuing data records that now range from 23 to 35 years. We evaluate chemistry on a weekly basis year-round at the small watershed-scale at BBWM, quarterly in LTM, and annually during the historical index period for the HELM lakes. TIME lakes were last sampled in 2016; current plans are to re-survey these lakes once during the next five-year project period. These project components provided a *statistical framework* for inferring regional patterns in chemistry using TIME and LTM (and ELS-II under separate funding). The *long-term records* of LTM, HELM and BBWM provide information on seasonal and annual variability, and thus provide a seasonal context for the annual surveys.

Expected Results. This information is needed for EPA to meet its Congressional mandate to assess the effectiveness of the CAAA. The combination of site-specific data within the regional context provides a rigorous assessment of the effects of declining pollutant emissions on SO_4 concentrations, base cation depletion, and changes in N-saturation or DOC contributions to acid-base status. The results are also central to assessing whether additional emission reductions may be needed to produce recovery.

Project Status: Water Chemistry

Field sampling. All project field objectives in 2017 were accomplished as planned. A summary of the annual field schedule for this project is provided below (Table 2). Funding cuts resulted in the elimination of sampling of the TIME lakes. HELM sampling is normally done by helicopter and due to funding the number of sites were cut from 25-30 to 9 for the 2nd year in a row and were done by hiking in via new project partner Julia Daly, UMaine-Farmington. However, a benefit to this new approach for HELM is that Daly has instrumented these lakes with temperature loggers, allowing for new research avenues regarding lake phenology.

Table 2. Annual project field schedule for lake sampling

Project	sub-project	n	Times Sampled	Field work	May	June	July	Aug.	Sept.	Oct.
RLTM-Maine										
	seepage	3	3	UMaine	X		X			X
	drainage	10	3	UMaine/UNH	X		X			X
	LTM lakes	3	1	UMaine					X	X
TIME										
	New England	31	0	UNH						
	Adirondacks	43	0	ALSC						
HELM		9	1	UMaine/UMF						X

Analytical. Analyses are complete for all samples collected through 2017. All laboratory analyses for LTM and HELM are conducted at the University of New Hampshire Water Quality Analysis Laboratory (WQAL) except for color and closed cell pH for RLTM samples (conducted at UMaine to meet holding time requirements) and aluminum. Total and organic aluminum samples are processed on an ICP at the USDA Forest Service Region 1 laboratory in Durham, NH. All analyses for RLTM and HELM continue to be conducted by, or under the supervision of, Jody Potter as has been the case since 2012.

Samples from East Bear Brook at BBWM, which are collected on a regular basis year-round, continue to be analyzed at the University of Maine Sawyer Water Research Lab. The LTM color and closed cell pH samples were also analyzed there.

The number of samples collected and processed over the last 5 years is 676, which includes all analytical analyses except the subset of lakes that are being analyzed for DOC quality (n = 400) and dissolved greenhouse gases (n = 221).

Data reporting. All data collected through 2016 have been delivered to EPA. The next delivery of data to EPA is expected before August 2018, after evaluation of inter-laboratory comparisons and regular QA analyses by UNH and UMaine, pending receipt of continued funding.

Presentation of findings. Several publications and presentations continue to result from this project and are listed at the end of this report. This project year, leveraged funding supported most of one M.S. thesis nearing completion (A. Gavin) and a Ph.D. dissertation nearing completion (K. Patel) at UMaine under the supervision of co-PI Nelson and BBWM PI Fernandez, respectively; results of those projects are now published (Gavin et al. 2018) or in review (Patel et al. 2018a, 2018b).

New developments. During the past five years we were able to make routine two new sets of analyses to continue to extract new and innovative information from these study sites. A subset of lakes were analyzed for DOC quality using SUVA and fluorescence (EEMS) analysis, and tested methods for measurement of dissolved greenhouse gases (CH_4, CO_2, and N_2O) in surface waters. Moving forward, these data will provide valuable insight into changes in organic sources to acid-base status as well as the influence of precipitation event variability on long-term changes in surface water chemistry. Graduate student Amanda Gavin (UMaine) instrumented a subset of LTM and HELM lakes with Hobo Temperature Loggers to support her research regarding how changing DOC concentrations could affect coldwater refugia for fish. Results of this investigation are currently in preparation for submission as a journal article and final M.S. thesis chapter, to be completed August 2018. Temperature loggers were re-deployed in several lakes to continue this investigation with funding that will begin in summer 2018.

Publications using related project information ***(2017-2018 publications in bold):***

Gavin, A.L., S.J. Nelson, A.J. Klemmer, I.J. Fernandez, K.E. Strock, W.H. McDowell, 2017. Climate linkages to increases of dissolved organic carbon in acid-sensitive high elevation lakes. Accepted; available online. Water Resources Research (2017WR020963).

Soranno, P.A., and 79 others, including S.J. Nelson, W.H. McDowell, C. Funk, J. Lynch, 2017. LAGOS-NE: A multi-scaled geospatial and temporal database of lake ecological context and water quality for thousands of U.S. lakes. GigaScience, gix101, https://doi.org/10.1093/gigascience/gix101

Nelson, S.J., Capone, S.B., Dukett, J.E., Houck, N. 2017. Lake Site Assessments: US EPA TIME-Adirondack Lakes. Report to US EPA and NYSERDA. (Report)

Brown, R.E., S.J. Nelson, J.E. Saros, 2016. Paleolimnological evidence of the consequences of recent increased dissolved organic carbon (DOC) in lakes of the northeastern USA. Journal of Paleolimnology, *57*(1), 19-35.

Crossman, J., M.C. Eimers, N.J. Casson, D.A. Burns, J.L. Campbell, G.E. Likens, M.J. Mitchell, S.J. Nelson, J.B. Shanley, S.A. Watmough, K.L. Webster, 2016. Regional meteorological drivers and long term trends of winter-spring nitrate dynamics across watersheds in north-eastern North America. Biogeochemistry, *130*(3), 247-265.

Boeff, K.A., K.E. Strock, J.E. Saros. 2016. Evaluating planktonic diatom response to climate change across three lakes with differing morphometry. *J Paleolimnol*. DOI 10.1007/s10933-016-9889-z

Strock, K.E., Saros, J.E., Nelson, S.J., S.D. Birkel, J.S. Kahl, W.H. McDowell. 2016. Extreme weather years drive episodic changes in lake chemistry: implications for recovery from sulfate deposition and long-term trends in dissolved organic carbon. *Biogeochemistry*, *127*(2-3), 353-365.

Strock, K., S. Nelson, J. Kahl, J. Saros, W. McDowell, 2014. Decadal trends reveal recent acceleration in the rate of recovery from acidification in the northeastern US. Environ. Sci. Technol. 48(9):4681-4689.

Sanclements, M., G. Oelsner, D. McKnight, S.J. Nelson, J. Stoddard, 2012. New insights into the source of decadal increases of dissolved organic matter (DOM) in acid-sensitive lakes of the northeastern U.S. Environmental Science and Technology 46(6): 3212–3219; DOI: 10.1007/s11356-009-0176-7.

Nelson, S.J., P. Vaux, M.J. James-Pirri, and G. Giese. 2012. Natural resource condition assessment: Cape Cod National Seashore, Massachusetts. Natural Resource Report NPS/NER/NRR—2012/605. National Park Service, Fort Collins, Colorado.

James-Pirri, M. J., S. J. Nelson, and P. D. Vaux. June 2011. Natural Resource Condition Assessment for Saugus Iron Works National Historic Site. Natural Resource Report NPS/NER/NRR—2011/457. National Park Service. Fort Collins, Colorado.

Kerr, J.G., M.C. Eimers, I.F. Creed, M.B. Adams, F. Beall, D. Burns, J.L. Campbell, S.F. Christopher, T.A. Clair, F. Courchesne, L. Duchesne, I. Fernandez, D. Houle, D.S. Jeffries, G.E. Likens, M.J. Mitchell, J. Shanley, H. Yao, 2011, The effects of seasonal drying on sulphate dynamics in streams across southeastern Canada and the northeastern USA, Biogeochemistry DOI 10.1007/s10533-011-9664-1.

Navrátil, T., S.A. Norton, I.J. Fernandez, S.J. Nelson, 2010. Twenty-year inter-annual trends and seasonal variations in precipitation and stream water chemistry at the Bear Brook Watershed in Maine, USA. Environ. Monit. Assess. 171:3-21.

Norton, S.; Fernandez, I.; Kahl, J.; Rustad, L.; Navratil, Tomas; Almquist, H., 2010. The evolution of the science of Bear Brook Watershed in Maine, USA. Environmental Monitoring and Assessment, 171(1-4): 3-21.

Vaux, P.D., S.J. Nelson, N. Rajakaruna, G. Mittelhauser, K. Bell, B. Kopp, J. Peckenham, G. Longsworth, 2008. Assessment of natural resource conditions in and adjacent to Acadia National Park, Maine. Natural Resource Report NPS/NRPC/WRD/NRR—2008/069. National Park Service, Fort Collins, Colorado.

Baumann, A.J. and J.S. Kahl, 2007. Chemical trends in Maine High Elevation Lakes. LakeLine 27:30-34.

Hunt, K., J.S. Kahl, J. Rubin, and D. Mageean, 2007. Assessing the science-based needs of stakeholders; a case study on acid rain research and policy. Journal of Contemporary Water Research and Education, 136: 68-79.

Rosfjord, C., K. Webster, J.S. Kahl, S.A. Norton, I. Fernandez, and A. Herlihy, 2007. Anthropogenically-driven changes in chloride complicate interpretation of base cation trends in lakes recovering from acidic deposition. Environ Sci Technol, 41:7688 -7693.

Rosfjord, C., J.S. Kahl, K. Webster, S. Nelson, I. Fernandez, L. Rustad, and R. Stemberger, 2006. Acidic deposition-relevant changes in lake chemistry in the EPA Eastern Lake Survey, 1984-2004. Final report to USDA NSRC, Durham, NH. 69 p.

Campbell, J, J. Hornbeck, M. Mitchell, M. Adams, M. Castro, C. Driscoll, J.S. Kahl, and others, 2004. Input-output budgets for inorganic nitrogen for 24 watersheds in the northeastern United States. Water Air Soil Pollution, 151:373-396.

Lawler, J., J. Rubin, B.J. Cosby, I. Fernandez, J.S. Kahl, S. Norton, 2005. Predicting recovery from acidic deposition: Applying a modified TAF (Tracking Analysis Framework) Model to Maine High Elevation Lakes, Water Air Soil Pollution. 164:383-389.

Dupont, J., T. Clair, C. Gagnon, D. Jeffries, J.S. Kahl, S. Nelson, and J Peckenham, 2005. Estimation of critical loads of acidity in the northeastern US and eastern Canada. Environ. Monit. Assess. 109:275-291.

Kahl, J.S., J. Stoddard, R. Haeuber, S. Paulsen, R. Birnbaum, F. Deviney, D. DeWalle, C. Driscoll, A. Herlihy, J. Kellogg, P. Murdoch, K. Roy, W. Sharpe, S. Urquhart, R. Webb, and K. Webster, 2004. Response of surface water chemistry to changes in acidic deposition: implications for future amendments to Clean Air Act. Environmental Science and Technology, Feature Article 38:484A-490A.

Norton, S., I. Fernandez, J.S. Kahl, and R. Reinhardt, 2004. Acidification trends and the evolution of neutralization mechanisms through time at the Bear Brook Watershed, Maine, USA. Water, Air, Soil, Pollution Focus 4:289-310.

Dissertations/theses:

Gavin, A.L. 2018 (expected). Physical & chemical response of small, north temperate lakes to recovery from acidification & climate change M.S. Thesis, University of Maine, Orono, Maine.

*** Patel, K.F. 2018 (expected). Nitrogen cycling during a period of environmental change. Ph.D. Dissertation, University of Maine, Orono, Maine.**

Boeff, K. 2014. Evaluating the effect of a changing climate on thermocline depth in Maine's Great Ponds. Master's thesis, University of Maine, Orono, Maine.

Brown, R. 2014. Assessing the ecological effects of increased dissolved organic carbon in Maine lakes over recent decades. Master's thesis, University of Maine, Orono, Maine.

Strock, K.E. 2013. Deciphering Climate-Mediated Changes in Boreal Lake Ecosystems. Ph.D. Dissertation, University of Maine, Orono, Maine.

* Not directly funded by this project but uses Bear Brook data and an LTM co-PI is on committee

Presentations using related project information ***(recent presentations in bold)****:*

Gavin, A., 2018. Physical & chemical response of small, north temperate lakes to recovery from acidification & climate change. University of Maine College of Natural Sciences, Forestry, and Agriculture Graduate Student Awards Competition, March 9, 2018. *Award-winning presentation; George F. Dow Graduate Scholarship.*

Gavin, A.L., S. J. Nelson, I.J. Fernandez, J.E. Saros, A. J. Klemmer, K.E. Strock, McDowell, W.H., 2018. Assessing Dissolved Organic Carbon Trends in Maine Lakes. Maine Water Conference, Augusta, ME, March 29, 2018.

Nelson, S.J., C.Y. Chen, J.S. Kahl, 2017. Water chemistry matters: Mercury bioaccumulation in dragonfly larvae bio-sentinels of northeastern lakes is correlated with Al, pH, DOC, and lake morphology. BIOGEOMON 2017, The 9th International Symposium on Ecosystem Behavior. August 20-24, 2017. Litomyšl, Czech Republic.

Saros, J.E., W. Gawley, S.J. Nelson, A. Klemmer, 2017. Monitoring Dissolved Organic

Carbon (DOC) and Water Clarity in Maine's Lakes. First Annual Lake Monitoring Summit, Feb. 15, 2017. Augusta, ME.

Contosta, A., N. Casson, S. Garlick, S. Nelson, 2017. Winter Climate Change in the Northern Forest: Scientific Synthesis and Management Implications. 2017 Conference of the Northeastern Ecosystem Research Cooperative (NERC). Saratoga Springs, NY, March 28-29, 2017. (Poster)

Gavin, A.L., S.J. Nelson, I.J. Fernandez, K.E. Strock, A. Klemmer. 2017. Climate linkages to increases of dissolved organic carbon in acid-sensitive high-elevation lakes. BIOGEOMON 2017, The 9th International Symposium on Ecosystem Behavior. August 20-24, 2017. Litomyšl, Czech Republic. (Poster)

Nelson, S.J., C.Y. Chen, D.P. Krabbenhoft, J.S. Kahl. 2016. Beyond "Hotspots": Dragonfly BioSentinels Describe Vulnerability (or not) of Northeastern Lakes and Their Foodwebs to Mercury Accumulation. 2016 Conference of the New England Association of Environmental Biologists (NEAEB), March 23-25, 2016, Rockport, ME.

W.H. McDowell, S.J. Nelson, J.D. Potter, 2015. DOC concentrations of New England (USA) lakes: is there a response to changing atmospheric deposition? Acid Rain 2015, Rochester, NY, Oct. 19–23, 2015.

Roy, K., H. Pembrook, S. Nelson, A. Riscassi, M. McHale, E. Boyer, G. Lampman, C. Funk, 2015. Long Term Monitoring of Acidification in Sensitive Areas of the Northern and Eastern United States: A New Generation of Research. Poster Presentation. Acid Rain 2015, Rochester, NY, Oct. 19–23, 2015.

McDowell, W.H. 2015. EPA TIME/LTM New England 2015. EPA Clean Air Act Cooperators meeting, Montpelier, VT. May 26 2015.

McDowell, W.G., K. Webster, S.J. Nelson, W.H. McDowell, J. Haney. Regulation and results: biotic and abiotic changes to northeastern lakes following tightening of air emission rules. Society for Freshwater Science, Milwaukee, WI, May 17- 21, 2015.

Appling, A.P., W.H. McDowell, J.D. Potter, S.J. Nelson, J.S. Kahl, 2014. From the frying pan into the fire? Lake greenhouse gas responses to acid rain recovery. Joint Aquatic Sciences Meeting. Portland, OR, May 18 – 23, 2014.

Brown, R.E., Saros, J.E. & S.J. Nelson. 2014. Algal community response to increases in dissolved organic carbon over recent decades. Poster presentation. Association for the Sciences of Limnology & Oceanography, Portland, OR, May, 2014.

Boeff, K. & J.E. Saros. 2014. Evaluating the effect of a changing climate on thermocline depth in Maine's Great Ponds. Poster presentation. Association for the Sciences of Limnology & Oceanography, Portland, OR, May, 2014.

Brown, R.E., Saros, J.E. & S.J. Nelson. 2014. Algal community response to increases in dissolved organic carbon over recent decades. Poster presentation. Maine Water Conference, Augusta, ME, March, 2014.

Strock, K.E., Saros, J.E., Nelson, S.J. & S. Birkel. 2014. Interactive effects of extreme weather and reduced sulfate deposition: accelerated recovery from acidification and increased brownification in lakes of the Northeast U.S. Association for the Sciences of Limnology & Oceanography, Portland, OR, May, 2014.

Boeff, K. & J.E. Saros. Evaluating the effect of changing wind strength on thermocline depth in Maine's Great Ponds. 22nd Annual Harold W. Borns Jr. Symposium, Orono, ME, USA, April, 2014.

Brown, R.E., Saros, J.E. & S.J. Nelson. 2014. Algal community response to increases in dissolved organic carbon over recent decades. 22nd Annual Harold W. Borns Jr. Symposium, Orono, ME, USA, April, 2014.

S.J. Nelson, 2013. School of Forest Resources Faculty Blitz. Sept. 13, 2013.

Boeff, K. & J.E. Saros. 2013. Evaluating the effect of a changing climate on thermocline depth in Maine's Great Ponds. Poster presentation. North American Diatom Symposium, Bar Harbor, ME, August, 2013.

Brown, R.E., Saros, J.E. & S.J. Nelson. 2013. Algal community response to increases in dissolved organic carbon: Implications for drinking water utilities. Poster presentation. North American Diatom Symposium, Bar Harbor, ME, August, 2013.

Nelson, S.J., C. Chen, D.P. Krabbenhoft, J.S. Kahl, B. Zoellick, 2013. Validating landscape models for mercury in northeastern US lakes using dragonfly larvae as mercury bio-sentinels. Accepted for poster presentation at the ICMGP - International Conference on Mercury as a Global Pollutant, July 28- Aug. 3, 2013, Edinburgh, Scotland.

Boeff, K., J. Saros. 2013. Evaluating the Effect of Changing Wind Strength on Thermocline Depth in Maine's Great Ponds. 21st Annual Harold W. Borns Jr. Symposium, Orono, ME, USA, April, 2013.

Brown, R.E., J.E. Saros, S.J. Nelson. Algal community response to increases in dissolved organic carbon in Maine lakes: implications for drinking water utilities. 21st Annual Harold W. Borns Jr. Symposium, Orono, ME, USA, April, 2013.

Strock, K.E., J.E. Saros, S. Birkel, S.J. Nelson, 2013. Exploring the effects of extreme hydrologic events in the northeastern U.S.: Implications for brownification and episodic acidification in Maine Lakes. 21st Annual Harold W. Borns Jr. Symposium, Orono, ME, USA, April, 2013.

Nelson, S.J., C. Chen, D.P. Krabbenhoft, J.S. Kahl, 2013. Dragonfly larvae as mercury bio-sentinels: a statistical survey of northeast lakes reveals landscape-driven patterns in water and biota mercury concentrations. NERC (Northeastern Ecosystems Research Cooperative) meeting, March 19 – 20, 2013, Saratoga Springs, NY.

Strock, K.E.D., J.E. Saros, S.J. Nelson. 2013. The effects of extreme climate events on lakewater chemistry: Implications for "brownification" in Maine lakes. Maine Water Conference. Augusta, Maine, March 19, 2013.

Strock, K.E.D., J.E. Saros, S.J. Nelson, S.D. Birkel. 2013. The effects of extreme climate events on lakewater chemistry: implications for dissolved organic carbon trends in the northeast U.S. American Society of Limnology and Oceanography Meeting. New Orleans, Louisiana, February 17-22, 2013.

Sanclements, M., G. Oelsner, D. McKnight, I.J. Fernandez, S.J. Nelson, M.B. Adams, M. Mineau, K. Simon, 2012. The effects of acidification and recovery on DOM quality and source in temperate forested watersheds. BIOGEOMON 2012, July 15-20, 2012, Northport, ME.

Strock, K.E., J.E. Saros, S.J. Nelson, 2012. Analyzing Legacy Data in a Climate Context to Decipher Modern Changes in Lakewater Chemistry. Poster presentation. BIOGEOMON 2012, July 15-20, 2012, Northport, ME.

Fernandez, I.J., Norton, S.A., Nelson, S.J., Salvino, C., 2012. Evidence of Transient Alteration of N Dynamics From an Ice Storm at the Bear Brook Watershed in Maine, USA. Poster presentation. BIOGEOMON 2012, July 15-20, 2012, Northport, ME.

Saros, J.E., K.E.D. Strock, S. Birkel & S.J. Nelson. 2012. Deciphering the effects of extreme hydrologic events on the response of northeastern lakes to reduced sulfur deposition. 20th annual Harold W. Borns Symposium, University of Maine.

Nelson, S.J., J.S. Kahl, A.J. Baumann, K.B. Johnson, 2012. "Rugged shores and clear waters": Interpreting biogeochemical response to environmental stressors using the lakes and ponds of Maine's Baxter State Park. Maine Water Conference, Augusta, ME, March 14, 2012.

Strock, K.E., J.E. Saros, S. Nelson. Why climate matters in recovery from acidification in northeastern US surface waters. Maine Water Conference, Augusta, ME, March 14, 2012.

Baumann, A.J., J.S. Kahl, T.R. Boucher, S.J. Nelson, and K.J. McGuire, 2012. "Changes in surface water chemistry in Maine high elevation lakes in response to the 1990 Clean Air Act Amendments. Maine Water Conference, Augusta, ME, March 14, 2012.

Mineau, M. M., K. S. Simon, D. T. Ely; R. L. Rancatti, I. J. Fernandez, S. A. Norton, and H. M. Valett. 2011. Effects of chronic nitrogen enrichment and acidification on coupled nitrogen and phosphorus cycling in streams: Insights from multiple spiraling techniques. Annual meeting, North American Benthological Society, Providence, RI.

Nelson, S.J., P. Vaux, M.J. James-Pirri. Data-driven assessments of National Park resources. (Invited). Acadian Internship in Regional Conservation and Stewardship, July 15, 2011.

Schneider, S.B., I.J. Fernandez, S.A. Norton, K.S. Simon. 2011. Soil base cation response to two decades of change at the Bear Brook Watershed in Maine. Gordon Conference on Catchment Science: Interactions of Hydrology, Biology and Chemistry. Bates College, Lewiston, Maine. July 10-15.

Nelson, S.J., C. Chen, H. Roebuck, B. Zoellick. Sensible sentinels: Preliminary mercury data for dragonfly nymphs (*Odonata: anisoptera*) across northern New England corroborate expected spatial pattern. The 10th International Conference on Mercury as a Global Pollutant (ICMGP), Halifax, NS, July 24-29, 2011; and presented at the Acadia Science Symposium, October 26, 2011.

Baumann, A.J., and J.S. Kahl, 2009. Assessing the effectiveness of federal acid rain policy using remote and high elevation lakes in northern New England. North American Lake Management Society International Symposium, Hartford, CT, October 29, 2009.

Kahl, J.S., 2009. Changes in base cations related to long-term changes in Cl distribution in northeastern lakes. Gordon Research Conference, Forested Catchments, July 12-17, 2009, Proctor Academy, NH.

Kahl, J.S., 2008 (invited). Twenty year changes in spatial patterns of Cl distribution in the northeastern US. NH Water Conference, April, 2008.

Kahl, J.S., 2007 (invited). Using societal-based incentives to address new threats to New England Lakes. Day-long short course in New England Lake Science Academy, Camp Kieve, Maine. July, 2007.

Kahl, S., K. Webster, D. Sassan, C. Rosfjord, S. Nelson, M. Greenawalt-Yelle, 2007. Increasing Cl in northeastern surface waters: an indicator of increasing development pressure. Maine Water Conference, Augusta, ME, March 21, 2007.

Kahl, J.S. 2006 (invited). Acid rain in New England: using high elevation lakes as sentinels of change. Maine Mountain Conference, October 21, 2006. Rangeley, Maine

Kahl, J.S., *et al*., 2006 (invited). The design of a national mercury monitoring network: Learning from the EPA acid rain experience. The Eighth International Mercury Conference, Madison WI, August 8, 2006.

Kahl, J.S. *et al*., 2006. Obfuscation of trends in base cations by regional salt contamination. Hubbard Brook Committee of Scientists annual meeting, July 12, 2006.

Kahl, J.S., 2006 (invited). 'Natural and human-derived sources of acidity in Maine Atlantic Salmon Rivers'. Atlantic Salmon Commission workshop on acidity, Bangor ME. April 10, 2006.

Kahl, J.S., 2005 (invited). The intersection of environmental science and environmental policy. NH Charitable Foundation Lakes Region annual meeting, Meredith, NH, September, 2005.

Kahl, J.S., 2005 (invited). Tracking response and recovery in surface waters in the northeastern US. Annual meeting of the Ecological Society of America, Montreal, August, 2005.

Kahl, J.S., and Catherine Rosfjord, 2005 (invited). Acid rain and the Clean Air Act in the northeastern US. Annual meeting of the NH-ME Androscoggin River Watershed Council, Bethel, June, 2005

Kahl, J.S., 2005 (invited). Developing a lake research agenda for NH. NSF workshop on lake research infrastructure in the northeast, Colby Sawyer College, April 2005.

Kahl, J.S., S. Nelson, and A. Grygo, 2004. Surface water chemistry data for the northeastern US for interpreting climate and acid rain trends. Northeast Ecosystems Research Consortium meeting, Durham, NH, October, 2004.

Kahl, J.S., K. Webster, M. Diehl, and C. Rosfjord, 2004. Successes of the Clean Air Act Amendments of 1990. Maine Water Conference invited plenary talk, Augusta, ME, 2004.

Kahl, J.S. and K. Johnson, 2004. Acid-Base Chemistry and Historical Trends in Downeast Salmon Rivers. Maine Water Conference, Augusta ME, April 2004.

Kahl, J.S., 2004 (invited). The Clean Air Act Amendments of 1990; testing a program designed to evaluate environmental policy. Lecture, Colby College. April, 2004

S.J. Nelson, J.S. Kahl, N.C. Kamman, D.P. Krabbenhoft, W.H. Halteman, 2009. (Poster) Predicting mercury concentrations in northeast lakes using hydrogeomorphic features, landscape setting and chemical co-variates. Gordon Research Conference, Forested Catchments, July 12-17, 2009, Proctor Academy, NH.

Nelson, S.J., I. Fernandez, S. Norton, B. Wiersma, L. Rustad, J.S. Kahl, 2008. The Bear Brook Watershed in Maine: Long-term research supporting climate change inquiry. Hydroclimatic effects on ecosystem response: participant workshop, Syracuse, NY, September 19, 2008.

Nelson, S.J., N. Kamman, D. Krabbenhoft, J.S. Kahl, K. Webster, 2008. Evaluating spatial patterns in mercury and methyl mercury in northeastern lakes: Landscape setting, chemical climate, and human influences. Northeastern Ecosystem Research Cooperative Conference, Durham, NH, November 12-13, 2008.

Nelson, S.J. 2008. Evaluating spatial patterns in mercury and methyl mercury in northeastern lakes: landscape setting, chemical climate, and human influences. Maine Water Conference, Augusta, ME, March 19, 2008.

Recent Bear Brook publications and presentations that include "base program" data (East Bear Brook stream chemistry partly funded through this grant):

Patel K.F., Nelson S.J., Spencer C.J. & Fernandez I.J. Fifteen-year record of soil temperature for the Bear Brook Watershed in Maine. 2018a. Manuscript in revision. *Scientific Data.*

Patel K.F. & Fernandez I.J. Long-term N mineralization during three decades of N enrichment at the Bear Brook Watershed in Maine, USA. 2018b. Manuscript in review. *Environmental Monitoring and Assessment.*

Norton, S.A., I.J. Fernandez, and S.J. Nelson. 2017. Stream cation and Al trajectories during acidification and recovery at the Bear Brook Watershed in Maine, USA. BIOGEOMON 2017, The 9th International Symposium on Ecosystem Behavior. August 20-24, 2017. Litomyšl, Czech Republic.

Fatemi, F. R., Fernandez, I. J., Simon, K. S., & Dail, D. B. (2016). Nitrogen and phosphorus regulation of soil enzyme activities in acid forest soils. *Soil Biology and Biochemistry*, *98*, 171-179.

Phelan, J., Belyazid, S., Jones, P., Cajka, J., Buckley, J., & Clark, C. (2016). Assessing the Effects of Climate Change and Air Pollution on Soil Properties and Plant Diversity in Sugar Maple–Beech–Yellow Birch Hardwood Forests in the Northeastern United States: Model Simulations from 1900 to 2100. *Water, Air, & Soil Pollution*, *227*(3), 1-30.

Lawrence, G. B., Hazlett, P. W., Fernandez, I. J., Ouimet, R., Bailey, S. W., Shortle, W. C., ... & Antidormi, M. R. (2015). Declining Acidic Deposition Begins Reversal of Forest-Soil Acidification in the Northeastern US and Eastern Canada. *Environmental science & technology*, *49*(22), 13103-13111.

Eimers, C., J. Crossman, N. Casson, D. Burns, J. Campbell, G. Likens, M. Mitchell, S. Nelson, J. Shanley, S. Watmough, K. Webster, 2015. Nitrogen dynamics in the dormant season: an inter-watershed comparison. Poster presentation. Acid Rain 2015, Rochester, NY, Oct. 19–23, 2015.

Mineau M. M., F. R. Fatemi, I. J. Fernandez and K. S. Simon. 2014. Microbial enzyme activity at the watershed scale: Response to chronic nitrogen deposition and acute phosphorous enrichment. Biogeochemistry 117:131-142.

Mineau, Madeleine M., Chad M. Grigsby, Damon T. Ely, Ivan J. Fernandez, Stephen A. Norton, Tsutomu Ohno, H. Maurice Valett, and Kevin S. Simon. 2013. Chronic catchment nitrogen enrichment and stoichiometric constraints on the bioavailability of dissolved organic matter from leaf leachate. Freshwater Biology 58:248-260.

Gruselle, Marie-Cecile, Cayce Salvino, Ivan J. Fernandez, Kevin Simon and Corianne Tartariw. 2013. Does Chronic N Fertilization Increase P Limitation in Northeastern U.S. Forest Soils? (Paper 383-4). Presented at the ASA-CSSA-SSSA International Meetings. Tampa, Florida. November 3-6. ASA, CSSA, SSSA, Madison, WI.

Fatemi, Farrah, Michael D. SanClements, and Ivan J. Fernandez. 2013. Microbial Carbon Cycling Along a Drainage Sequence in a New England Forested Watershed. (Paper 252-2). Presented at the ASA-CSSA-SSSA International Meetings. Tampa, Florida. November 3-6. ASA, CSSA, SSSA, Madison, WI.

Morse, Jennifer L., Jorge Duran, Fred Beall, Irena Creed, Eric Enanga, Ivan Fernandez, and Peter M. Groffman. 2013. Soil denitrification fluxes and oxygen dynamics in three contrasting northeastern North American forests. Ecological Society of America. Minneapolis, Minnesota 8/4-8/9.

SanClements, Michael, Ivan Fernandez, Mary Beth Adams, and Jenny Erwin. 2013. Linking Stream DOM Quality and Source to Watershed Acidification and Recovery in Temperate Forests of the Northeastern United States. 12th North American Forest Soils Conference, Whitefish, Montana. p. 112.

Gruselle, Marie-Cecile, Ivan Fernandez, and Corianne Tatariw. 2013. Manganese Dynamics in the Third Decade of Forest Ecosystem Experimental Acidification and Nitrogen Enrichment. 12th North American Forest Soils Conference, Whitefish, Montana. p. 35

MacRae, J.D., C. Tatariw, D. Rothenheber, S. Nelson, I.J. Fernandez. The effects of nitrogen enrichment on forest soil microbial communities and their activities, 2013 AEESP 50th Anniversary Conference, July 14-16, Golden, CO.

Boca, Antra, Mercedes Roman Dobarco, Helga Van Miegroet, Marie-Cecile Gruselle, Beate Michalzik, and Ivan Fernandez. 2013. Linking Overstory, Soil and Climate Characteristics to Explain C Storage in Forest Soils. 12th North American Forest Soils Conference, Whitefish, Montana. p. 29.

Gruselle, M.-C., Fernandez, I., Simon, K., Norton S., 2013. The effects of long-term ecosystem N enrichment on 15N dynamics at the Bear Brook Watershed in Maine. NERC 2013 Conference, Saratoga Springs, NY.

Minocha, Rakesh, Swathi A. Turlapati, Stephanie Long, Mohammad M. Bataineh, Aaron Weiskittel, Ivan Fernandez, and Lindsey Rustad. 2013. Chronic N and S additions impact foliar physiology of forest trees at the Bear Brook Watershed in Maine, USA. Hubbard Brook Experimental Forest Annual Meeting, Thornton, NH.

Ohno, Tsutomu, Ivan J. Fernandez, Rachel L. Sleighter, and Patrick G. Hatcher. Influence of depth on soil organic matter characteristics: An ultrahigh resolution mass spectrometry study. Advanced Analytical Characterization of Natural Organic Matter, Goldscmidt 2013, Florence, Italy, August 25-30, 2013.

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Salt and Streams: Assessing ecological stress in New Hampshire watersheds at community, population, and molecular levels

Basic Information

Title:	Salt and Streams: Assessing ecological stress in New Hampshire watersheds at community, population, and molecular levels
Project Number:	2016NH201B
Start Date:	3/1/2016
End Date:	2/28/2018
Funding Source:	104B
Congressional District:	NH-02
Research Category:	Biological Sciences
Focus Categories:	Water Quality, Ecology, Methods
Descriptors:	None
Principal Investigators:	Amy Marie Villamagna

Publications

There are no publications.

Salt and Streams: Assessing ecological stress in New Hampshire watersheds at community, population, and molecular levels

Problem

New Hampshire's climate is expected to resemble that of the US Mid-Atlantic by 2100 (USGCRP 2009). With this shift comes increased air temperatures, less snow pack, more ice storms, and more rain on snow events. From a freshwater ecology perspective, much of central and northern New Hampshire's streams are currently populated by coldwater species (e.g., Brook trout; Neils 2009). As a result of increasing air temperatures, stream temperature will likely increase; however, the increase will vary among streams (Kelleher et al. 2011). For many species, this thermal shift may be within their fundamental tolerance range (e.g., 21°C thermal maxima for Brook Trout), barring additional physiological stress. However, growth in development (e.g., roads, housing) and energy production (mining, fracking) in northeastern states is causing additional stress on freshwater biota (Van Meter et al. 2011, Kelting et al. 2012). Among emerging concerns are the short-term and cumulative impacts of thermal and salinity stress on freshwater resources and biota (Findlay and Kelley 2011, Cuffney et al. 2010, Van Meter et al. 2011, Dalinsky et al. 2014, Stitt et al. 2014).

Recently it has been reported that salts are infiltrating into subsurface flow and groundwater before reaching streams (Daley et al. 2009). The infiltration of salts into soil and retention in groundwater systems adds a lag to the emergence of salts in streams, elevating Cl concentrations into summer months (Williams et al. 2000, Findlay et al. 2011, Kelting et al. 2012). The impacts of thermal variability and salt loading on freshwater biota have garnered attention and study in northern states, but it remains unclear how the synergy of salt and thermal stressors impact biota across the community, population and molecular levels.

Traditionally, biotic response to water quality degradation is measured using broad-based community metrics (e.g., Simpson's Index of Diversity) and/or assessing populations of select bio-indicator species (e.g., EPT= the macroinvertebrate orders of Ephemeroptera, Plecoptera, and Tricoptera). Rapid biological assessments examine community composition and the presence of indicator species to assess overall stress (Friberg et al. 2011); however, these methods are largely reliant on the loss of individuals and/or species, which could have cascading effects on biodiversity and the ecological function of streams. In order to avoid the potentially cataclysmic effects of osmo-thermal stress on NH streams, we need studies that investigate the biotic response along a gradient of salt and thermal stress. However, to truly avert the loss of species and ecosystem function, we need to develop techniques that will provide an early-warning signal of ecosystems in jeopardy.

Objectives

The goal of this project was to enhance biomonitoring efforts and early detection of thermal and salt stress on stream biodiversity in New Hampshire. To achieve this, our objectives were to:

1) **Evaluate differences in stream macroinvertebrate communities along a thermal-salt stress gradient.** We assessed macroinvertebrate community composition in ten 1st to 4th order wadeable streams across NH that vary along a thermal-salt stress gradient (Figure 1).
2) **Evaluate sub-lethal osmotic stress in mayfly larvae** by quantifying HSP expression in mayfiles. This objective was pursued by first conducting in-lab salt exposure trials using nymphal mayflies to create salt-stress response curves. The in-lab exposure trials were

followed by snapshot expression profiles from field caught individuals. Mayflies are a sensitive, yet very important source of prey in northern streams; therefore, the development of stress protein expression metrics in mayfly nymphs holds promise as a sensitive, early stage, and rigorous measure of the biotic impacts of salt load on freshwater habitats (Bauernfeind and Moog 2000, DeJong et al. 2006).

3) **Compare and evaluate benthic macroinvertebrate sampling techniques and potential indicator taxa for salt stress.** The NH Department of Environmental Services (NHDES)–Biomonitoring program has adopted a rock basket approach for assessing water quality using indicator taxa and community metrics. We set out to compare the rock basket approach to kicknetting over the months of May – October to evaluate their ability to detect small changes in community composition that may be attributed to elevated salt or temperature.

Methods

Site selection

Field sites were selected by using GIS to overlay the LoVoTECS network of stream monitoring sites with fish sample sites between 2009 and 2015. From this subset of NH streams, we selected sites based on median chloride concentrations derived from snapshot water chemistry data collected in May and July 2013 and July, Sept, Oct 2014. Our ten sites ranged

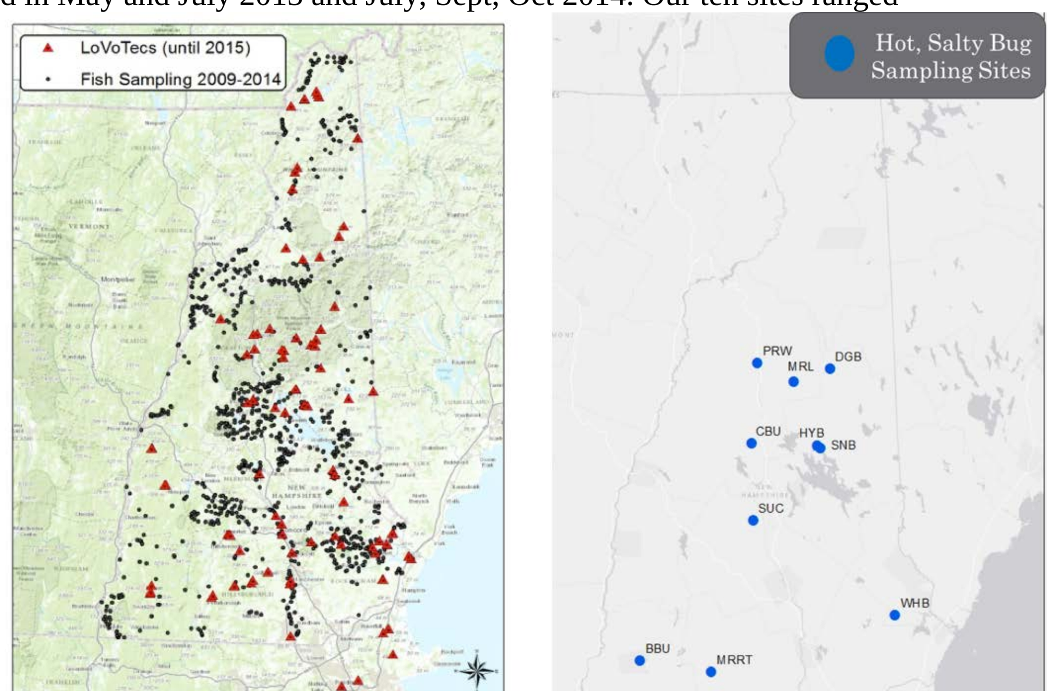

Figure 1: Network of LoVoTEC monitoring sites overlaid with NH Fish and Game Dept fish sampling sites between 2009-2014 (LEFT); Ten Hot, Salty Bugs sampling sites between May and October 2016.

from 4.35-52.6 mg/L of Chloride during this period. The ten sites represent a range of human impact; some sites have roads and development, and some sites have little to no human impact. Two of our sites, Mad River in Waterville Valley and Douglas Brook near the Kancamangus

Highway are located in the White Mountain National Forest. The other stream sites are located near minor and major road systems, with minimal to moderate influence from road salts and other anthropogenic influences. The ten sites include: Halfway Brook and Shannon Brook in Moultonborough, Mad River in Waterville Valley, Douglas Brook in Bartlett, Beaver Brook in Keene, Wednesday Hill Brook in Lee, Pemigewasset River in Woodstock, Clay Brook in Plymouth, Otter Brook in Peterborough, and Sucker Brook in Franklin.

Our research team adopted NAWQA and EPA Rapid Bio Assessment Macroinvertebrate Sampling protocols for multi-habitat kicknet sampling. We sampled each study stream once every month beginning in mid-May to September/October, 2016. At each site, we selected a 100-meter reach that was largely representative of the stream habitat. This 100-m reach was established in close proximity to continuously logging specific conductance, water temperature, and water level sensors; most sites consisted of sample reaches that were 50-meters upstream and 50-meters downstream, or, where that was not feasible, 25-meters and 75-meters. We sampled total of 10 kicks over the 100-meter stream reach, sampling different habitats in approximate proportion to their representation of the total surface area of the reach. We determined this by assigning a percentage of each habitat type (cobble, sand, or large woody debris) totaling 100%. In cobble substrate/habitat, we chose to kick in riffles or runs. In sand substrate and habitat, we mainly kicked in runs and slow moving water since that is the main stream morphology for this type of habitat. We placed all macroinvertebrates in labeled containers with 70% ethanol for preservation. If there were any predator macroinvertebrates, such as the family Corydalidae, then we used an additional container to store the predators.

In addition to kicknetting, we adopted the New Hampshire Department of Environmental Services (NHDES) biomonitoring program rock basket approach for macroinvertebrate sampling. At each site, we deployed 3 rock baskets side-by-side in a cobble and riffle habitat in close proximity to the continuously logging sensors. We collected rock baskets roughly every four weeks to collect macroinvertebrates from June to July for identification and enumeration. We left rock baskets in study streams for eight weeks from July/August to Septemeber/October to better compare results with NH DES Biomonitoring Program’s annual assessments. Our rock basket collection was similar to the NHDES sampling protocol, which included four, 5-gallon buckets, 3 of which will hold the rock baskets themselves, and one bucket to rinse and store the rocks that have been examined. We filled three buckets with stream water a quarter full and facing upstream with the opening facing towards the rock basket. One person lifted each basket into the bucket, making sure to catch any debris that comes loose from the basket. The research team thoroughly examined every rock in each basket, and the water in the bucket were filtered through a sieve. We placed all macroinvertebrates in rock basket labeled containers separately to the kick net samples, and stored in 70% ethanol to be preserved. We labeled containers with the correct site name and date sampled. Rocks were cleaned and put back into the baskets. The three baskets were then re-deployed in the same location.

Macroinvertebrate Identification

We preserved our field samples in 70% ethanol, transported them to the laboratory, and sorted and identified by family using NAWQA and EPA protocol for macroinvertebrate sorting and identification. References used to identify macroinvertebrates by family were from online sources from New England, and book sources, such as *A Guide to Common Freshwater Invertebrates of North America, An Introduction to the Aquatic Insects of North America 4th Edition, and Freshwater Macroinvertebrates of Northeastern North America.*

Community analysis

We calculated community composition metrics for each site visit, including: EPT family richness, % EPT, % Plecoptera, Philopotamidae (fingernet caddisfly; Tolerance Value: 0-4 according to NHDES) relative abundance, % Chironomidae (non-biting midges) and compared these metrics to the chloride concentration from the same sample visit. In addition, we used multiple linear regression to assess the relationship between the given community metric and a suite of potentially influential environmental factors: discharge, reach area, latitude, elevation, water temperature, pH, dissolved oxygen, as well as chloride and sodium. Finally, we explored the data using Canonical Correspondence Analysis to investigate the influence of chloride and other environmental conditions on community composition.

Salt exposure trials & HSP70 expression*:*

This portion go the student focused on three primary research objectives: 1) examine the concentration/distribution of HSP70 across the mayfly body; 2) quantify dosage-dependent response curves of HSP70 expression to gradients of sodium chloride using in-lab mesocosms; and 3) examine in-situ levels of HSP70 expression among mayflies in 10 New Hampshire streams across a chloride gradient (Figure 2). For the first objective, individuals were collected from nearby streams and dissected into four body regions: 1) head, 2) legs, 3) gills, and 4) abdomen. For salt trials, individuals were collected and transported to micro aquaria setups using one-liter beakers as tanks. The source water for micro aquaria originated from the site itself in order to keep baseline ionic conditions constant. Leaf pack was also collected from sites along with specimens to provide a substrate for attachment and a food source. To best mimic running water conditions, battery-powered bubblers were placed in each beaker to create an oxygenated environment. Specimens were exposed to a gradient of salt concentrations following a three-day acclimation period in order to rule out the possibility of stress protein expression due to handling/travel. Preceding salt dosage, several specimens were immediately extracted for proteins to provide a measure of baseline HSP70 expression. Applied concentrations of salt have included 150mg/L, 300mg/L, 400mg/L, 2000mg/L, and 4000mg/L; during these exposures, individuals were selected and proteins were extracted at the 1, 2, 4, and 168hr marks. In order to examine HSP70 levels across different field sites in NH, specimen collection occurred once per month May-September with a goal of no less than 15 specimens per site, per month. All protein extractions were carried out using physical homogenization coupled with T-PER extraction buffer. Quantification of proteins was then carried out by use of a bicinchoninin c acid assay (BCA) assay to determine the concentrations of total protein extracted via a nanodrop spectrophotometer. Finally, HSP70 expression was observed by western-blotting technique, exposing proteins separated by size (electrophoresis through a gel medium) to a primary monoclonal HSP70 antibody for specific binding of the protein of interest.

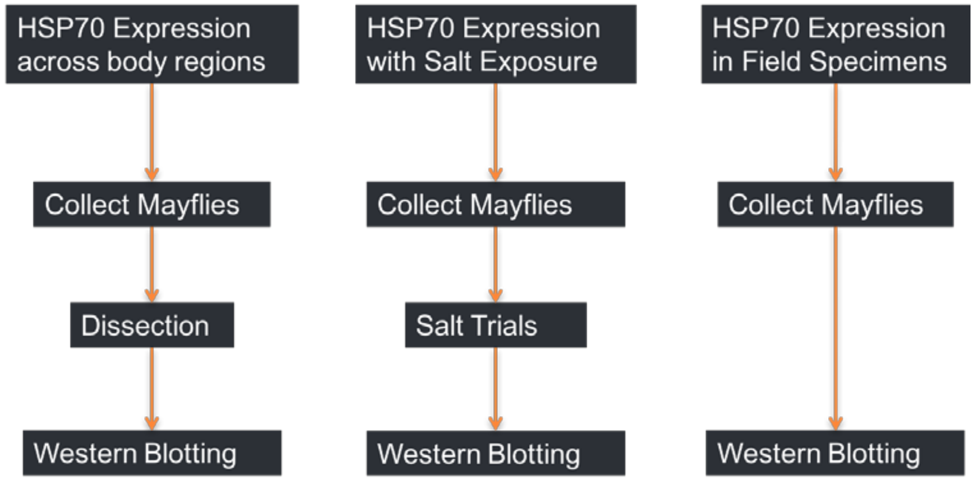

Figure 2: Overview of HSP70 analysis.

<u>Principal Findings & Significance</u>

Chloride concentrations

The summer of 2016 was an unusually dry season, leaving many of our study sites with water levels much lower than normal. Monthly snapshot water chemistry samples confirmed the initial classification of streams (based on snapshot sampling in 2013 and 2014). Our sites in southern NH (Keene and near Durham), generally had higher chloride concentrations that the other sites, but all sites had chloride levels below 60 mg/L (Figure 3). Even our highest chloride concentration was substantially below the EPA's chronic toxicity concentration of 230 mg/L. Chloride concentrations increased at most sites throughout the sampling season, which we believe is attributed to low water levels. Low surface water inflow suggests that groundwater likely comprised a larger portion of stream water. Thus, increasing concentrations throughout the summer may help support the findings of Daily et al. (2009).

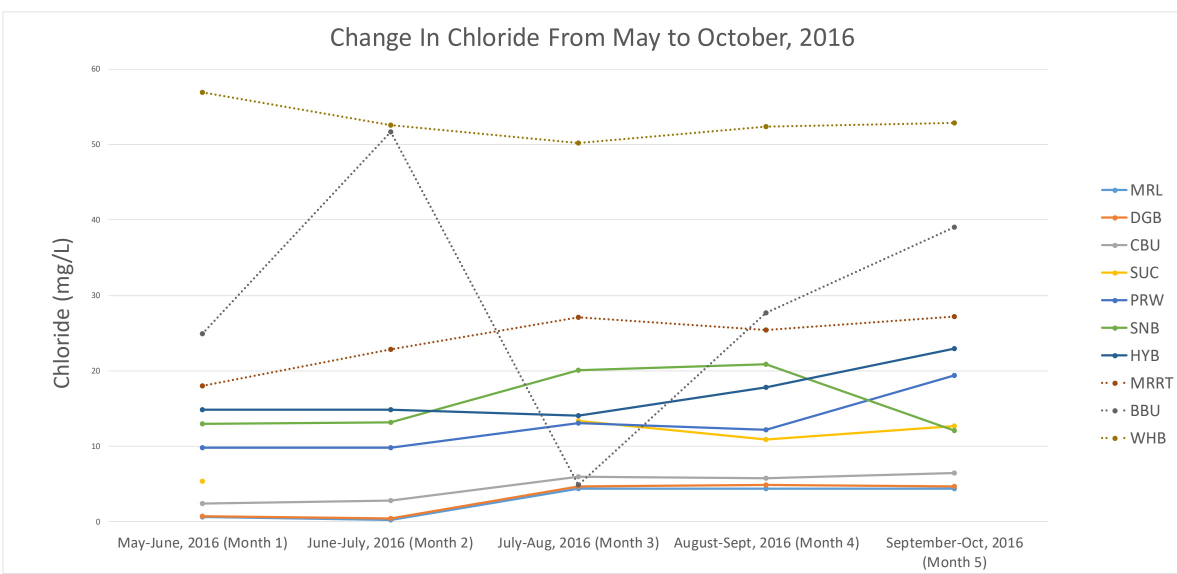

Figure 3: Snapshot sampling of chloride in 10 sample streams between May/June and September/October 2016.

Chloride and Community Composition

We found that chloride rarely explained a significant portion of the observed variation in the aforementioned community metrics used by NH DES Biomonitoring Program (Table 1 and graphs in Appendix A). We found the mean percent EPT at each site throughout the summer was inversely related to chloride; however, this relationship was weak and not statistically significant (Figure 4A-B). Interestingly, chloride was only a significant predictor of some metrics in late summer (late August through early October; Table 1). We also found an unexpected positive relationship between chloride concentration and the percent of the community comprised of Plecopterans. We are investigating this more to determine if the relationship was driven by a single family or if the unexpected positive relationship (more chloride, more Plecopterans) is consistent across the order. Either way, it is important to reconcile because percent of individuals from EPT orders are considered indicators of good water quality.

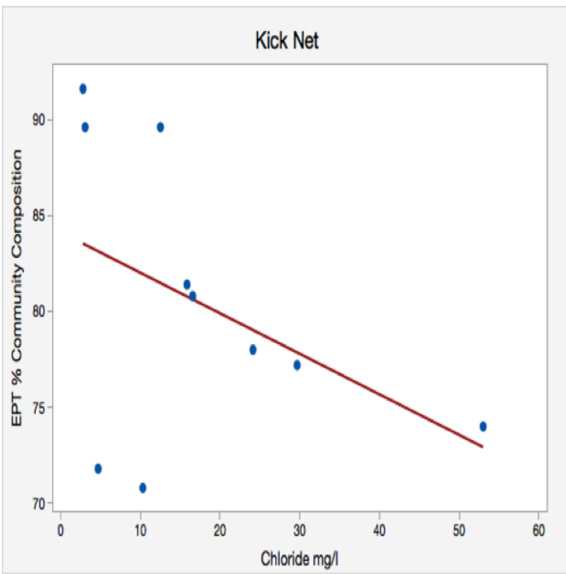

Figure 4A: Linear regression comparing average EPT % community composition and chloride for kick nets (r^2=.18, p=0.22)

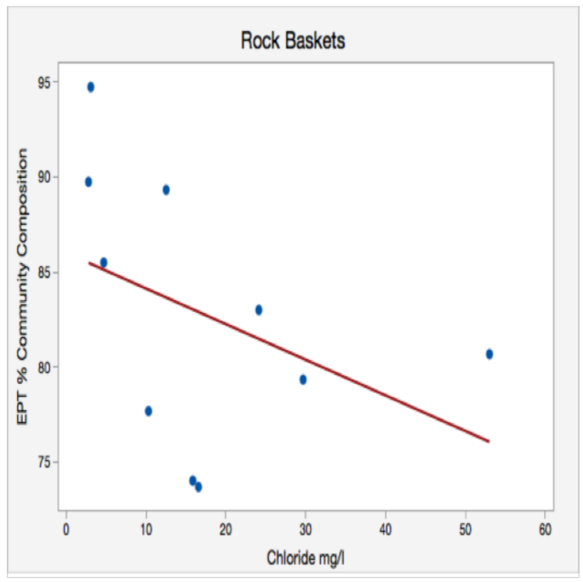

Figure 4B: Linear regression comparing average EPT % community composition and chloride for kick nets (r^2=.17, p=0.24)

Table 1: Summary of regression analyses of chloride and various macroinvertebrate community composition metrics.

	EPT Family Richness	% EPT	% Plecoptera	Philopotamid Abund.	% Chironomidae
Month	R^2	R^2	R^2	R^2	R^2
May-October	0.22*	0.11*	0.12	0.02*	0.03*
May-June	0.22*	0.00*	0.28	0.01	0.32*
June-July	0.07*	0.18*	**0.53**	0.14*	0.16*
July-August	0.13*	0.05*	0.09	0.00*	0.07
August-September	**0.53***	0.25*	0.06*	0.19*	**0.51***
September-October	**0.56***	**0.56***	0.01*	0.02*	0.25*
Bold= p< 0.05 and * means relationship was as hypothesized					

Relationship was as hypothesized and is significant

The lack of a clear and consistent relationship between chloride and the community metrics prompted us to take a multiple linear regression approach to better understand the influencers of the observed macroinvertebrate communities. We took a backwards parameter selection approach, starting with the full (global) model that included discharge, stream area, as well as snapshot measures of water temperature (snapshot), pH, dissolved oxygen, chloride, and sodium. Sample month was also included because there is uncertainty in the timing of emergence for all families observed. Table 2 below provides a summary of the best model for each community metric. Again, chloride was a significant predictor of Plecopteran abundance, but the relationship was unexpectedly positive. Water temperature was the factor most frequently included in significant best models. Month was also an important explanatory variable for percent Ephemeroptera and percent Tricoptera. We are in the process of investigating these patterns more thoroughly at the family level.

Table 2: Summary of the 'best' multiple linear regression models for each community composition metric.

Multiple Linear Regression Models to Predict Community Composition

Dependent variable	Independent variable	R^2	P value
% EPT	Elevation	0.19	<0.0001
	Sodium	0.11	0.004
% Ephemeroptera	Month	0.24	<0.0001
% Plecoptera	Chloride	0.30	<0.0001
% Tricoptera	Month	0.24	<0.0001
	Water Temperature	0.22	<0.0001
Total Family Richness	Water Temperature	0.13	0.005
	Latitude	0.16	0.002
Philopotamidae Relative Abundance	Water Temperature	0.24	0.001
Rhyacophilidae Relative Abundance	Month	0.11	0.044

The single best independent variable is listed first, followed by the second most, if applicable, by its unique variance (R^2) and p-value.

We have yet to find a consistent model that explains the individual community metrics discussed. However, many of these metrics are intended to be used as indicators of water quality rather than explicit measures of aquatic biodiversity. To better understand how chloride may be influencing community composition and structure, we conducted a series of Canonical Correspondence analyses (CCAs). CCAs are a multivariate approach to identify the suite of variables that best explain the composition and structure of a given community. We conducted CCA analysis for families within each Order separately because we did not have a large enough samples size to allow proper CCA for all families identified. Our results are preliminary at this time, and will be updated at the conclusion of this project.

Temperature and Community Composition

Using the continuously logging air and water temperature sensors, we were able to calculate the sensitivity of stream temperature to changes in air temperature at each study stream (i.e., stream thermal sensitivity). Sensitivity is reflected in the slope of the air to water temperature relationship. Sensitivity ranged between 0.41 and 0.68. This can be interpreted as for every degree (F) increase in air temperature there was an observed increase in stream temperature

Table 3 Summary of stream thermal sensitivity. to changes in air temperature.

Linear Regression - Mean Daily Water vs. Air		
Site	R sq	Slope
BBU	0.39	0.424
CBU	0.67	0.586
DGB	0.74	0.584
HYB	0.72	0.681
MRL	0.54	0.412
MRRT	0.60	0.520
PRW	0.88	0.509
SNB	0.70	0.612
SUC	0.78	0.605
WHB	0.95	0.506

between 0.41 and 0.68 degree (F). Table 3 provides a summary of the slope and r^2 values for each relationship. There was not a direct relationship between thermal sensitivity and chloride concentrations, suggesting other factors may be affecting stream temperature aside from roadway density within the stream's catchment.

The influence of stream temperature on macroinvertebrates was explored by focusing on the same key community composition metrics described for chloride analyses. We calculated the monthly mean, 7-day mean, and the mean daily max stream temperature in correspondence to the macroinvertebrate sampling events. Table 4 below provides the r^2 values for the linear regression between each as well as the slope of the relationship. Several of the observed linear relationships were significant, albeit weak, and for some metrics the relationship was positive suggesting that warmer temperatures were associated with greater proportional abundance of specified taxa (e.g. Corydalidae and Philopotamidae). We found no notable relationships between common diversity metrics, such as Simpson's Diversity, and stream temperature.

We also explored the relationship between each of the community metrics and the sensitivity of the stream to air temperature changes. The rationale for this analysis was that temperature sensitive streams may experience drastic diurnal and weekly temperature changes during summer months. These drastic temperature shifts can be a disturbance to some aquatic organisms. Table 5 below provides the r^2 values for the linear regression between each as well as the slope of the relationship. Interestingly, we found that the only community metrics to be significantly related to the thermal sensitivity of the stream were common diversity metrics Simpsons Index of Diversity and Shannon's Diversity Index, both of which were not significantly related to temperature itself. For both metrics, the relationship was negative suggesting that the greater thermal sensitivity the less diverse the community's composition of macroinvertebrates would be. We plan to explore this result more with more extensive family level analyses and multivariate approaches.

Table 4: Summary of the linear relationships between stream temperature and community composition metrics. Bolded values note statistical significance (p ≤ 0.05).

Explanatory Variable	**Monthly Mean**		**168 Hour (7 day) Mean**		**Average Daily Max**	
Response Variable	R-sq	Slope	R-sq	Slope	R-sq	Slope
% EPT	0.04	-0.673	0.07	-0.964	0.05	-0.726
% Plecoptera	**0.13**	**-1.673**	**0.13**	**-1.731**	**0.13**	**-1.643**
% Philopotamidae	**0.26**	**3.045**	**0.14**	**2.275**	**0.26**	**2.982**
% Leptophlebiidae	**0.27**	**-0.922**	**0.19**	**-0.739**	**0.27**	**-0.905**
% Corydalidae	**0.14**	**0.615**	**0.23**	**0.781**	**0.14**	**0.611**
Simpson's Index (1-D)	0.03	-0.005	0.01	-0.003	0.03	-0.005
Shannon's Index (H)	0.02	-0.013	0.00	-0.003	0.01	-0.010
Shannon's Evenness	0.06	-0.006	0.06	-0.006	0.07	-0.006

Table 5: Summary of the linear relationships between stream thermal sensitivity (measured as the linear slope between air and water temperatures (Table 3) and various community composition metrics.

Air vs. Water Slope Values as Explanatory Variable		
Explanatory Variable	**Slope**	
Response Variable	R-sq	Slope
% EPT	0.00	3.830
% Plecoptera	0.09	-22.290
% Philopotamidae	0.03	23.620
% Leptophlebiidae	0.02	5.473
% Corydalidae	0.01	3.663
Simpson's Index (1-D)	**0.15**	**-0.280**
Shannon's Index (H)	**0.14**	**-0.867**
Shannon's Evenness	0.00	-0.090

HSP Analysis

The first year of work has been largely devoted to developing a field to lab protocol for assessing HSP70 in macroinvertebrates, first with a focus on mayflies and later stoneflies. Thus far, the HSP70 stress response has been identified in both mayfly and stonefly nymphs across several regions of the body (Figure 5), as well as in whole insect based extractions. However, after little HSP70 expression was observed in several western blots (additional examples of HSP70 western blotting results are available in Appendix C) we have taken a series of approaches to rule out any possible researcher-based error. These included eliciting the HSP70 stress response, ensuring proteins were not degraded or aggregated prior to analysis, and exploring the possibility of minimal HSP70 expression being present. We are working through each step of the protocol to be sure that the method is appropriate and that the lack of HSP70 expression is a true result suggesting low stress environments for mayflies, rather than one influenced by procedural decisions or chemical choices.

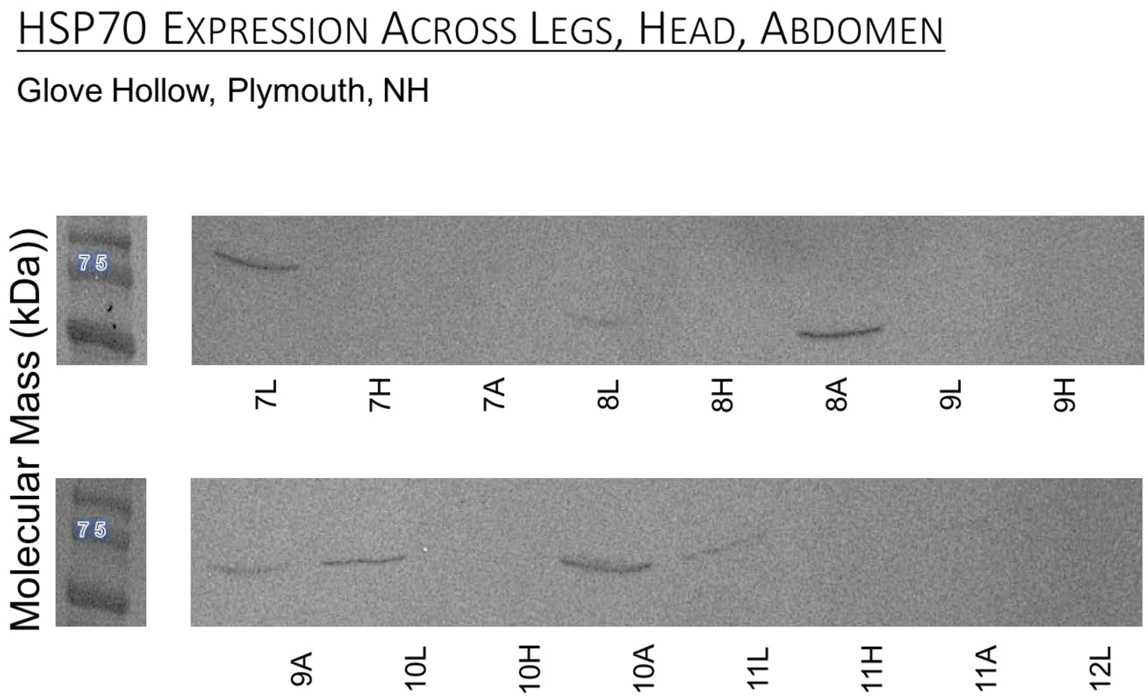

Figure 5: Western blot results of HSP70 expression across the legs, head, and abdomen of mayflies collected in spring 2016.

Recent western blots have begun to assert the possibility that this molecular response to salt stress may not reflect the true biological stress (or lack of stress) in the organisms. Current and future work involves pursuing longer lab exposures to mimic exposure to elevated salinity in the field (24hrs – 5 days), simultaneous exposures to different stressors (salt, heat, heat + salt), extractions of individuals at more frequent and longer time periods following exposure, and probing western blots with an additional HSP60 antibody (also known to be part of the stress response system). We will continue to explore differences in HSP expression across body parts. Work on the HSP70 protocol and lab trials continues and additional samples will be taken in summer 2017 for future snapshot assessment.

Future work:

This research will continue through summer of 2017 with funding from NH WRRC during which the same 10 sites will be revisited and the HSP70 lab protocol development and vetting will continue. HSP70 protocol will expand to include stoneflies and will focus on combining salt and thermal stress. We will specifically be examining interannual variability potential attributed to precipitation and stream water levels, which impact chloride concentrations and water temperature. Likewise, we will begin to assess the relationship between longer term exposure to elevated chloride and temperature using data from installed temperature and conductivity sensors.

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Notable Awards:

Dr. Amy Villamagna was honored with the Helen Abbott Endowed Professors of Environmental Studies (2016-2020) for her research on the environment and engagement of students in research.

Publications and presentations:

2016

Fruit, R. , A. Villamagna, B. O'Donnell. 2016. *Stress Protein Expression: An Early Warning Sign of Freshwater Community Degradation via Road Salt Runoff in New Hampshire* (poster), 2016 NH Water and Watersheds Conference in Plymouth, New Hampshire

Duquette, R., A. Villamagna, B. O'Donnell. 2016. *Assessment of Mayfly, Stonefly and Caddisfly abundances in relation to chloride in New Hampshire streams.* Hubbard Brook Research Experience for Undergraduates Symposium (Thorton, NH)

Lafortune, T., A. Villamagna, B. O'Donnell. 2016. *Air and Stream Temperature Relationships and Influence on Macroinvertebrate Communities in New Hampshire.* Hubbard Brook Research Experience for Undergraduates Symposium (Thorton, NH)

Duquette, R., A. Villamagna, B. O'Donnell. 2016. *Assessment of Mayfly, Stonefly and Caddisfly abundances in relation to chloride in New Hampshire streams.* EPSCoR Research for Undergraduates Symposium at University of New Hampshire (Durham, NH)

Lafortune, T., A. Villamagna, B. O'Donnell. 2016. *Air and Stream Temperature Relationships and Influence on Macroinvertebrate Communities in New Hampshire.* EPSCoR Research for Undergraduates Symposium at University of New Hampshire (Durham, NH)

2017

Duquette, R., A. Villamagna, B. O'Donnell. 2017. *Assessment of Mayfly, Stonefly and Caddisfly abundances in relation to chloride in New Hampshire streams.* New England Association of Environmental Biologists annual meeting (Hartford, CT) *[poster printed but not presented due to poor blizzard travel conditions]*

Lafortune, T., A. Villamagna, B. O'Donnell. 2016. *Air and Stream Temperature Relationships and Influence on Macroinvertebrate Communities in New Hampshire.* New England Association of Environmental Biologists annual meeting (Hartford, CT) *[poster printed but not presented due to poor blizzard travel conditions]*

Fruit, R. , A. Villamagna, B. O'Donnell. 2017. *Quantification of HSP70 Expression in Mayflies: A Novel Bioindicator of Road Salt Pollution.* New England Association of Environmental Biologists annual meeting (Hartford, CT) oral presentation

Mazzone, M. A. Villamagna, B. O'Donnell. 2017. *Assessing Salt Stress In Selected NH Streams at the Community Level For Macroinvertebrates.* New England Association of Environmental Biologists annual meeting (Hartford, CT) *[oral presentation prepared but not presented due to poor blizzard travel conditions]*

Duquette, R., A. Villamagna, B. O'Donnell. 2017. *Assessment of Mayfly, Stonefly and Caddisfly abundances in relation to chloride in New Hampshire streams.* Plymouth State University Showcase of Excellence (poster)

Lafortune, T., A. Villamagna, B. O'Donnell. 2016. *Air and Stream Temperature Relationships and Influence on Macroinvertebrate Communities in New Hampshire.* Plymouth State University Showcase of Excellence (poster)

Fruit, R. , A. Villamagna, B. O'Donnell. 2017. *Quantification of HSP70 Expression in Mayflies: A Novel Bioindicator of Road Salt Pollution.* Plymouth State University Showcase of Excellence (oral presentation)

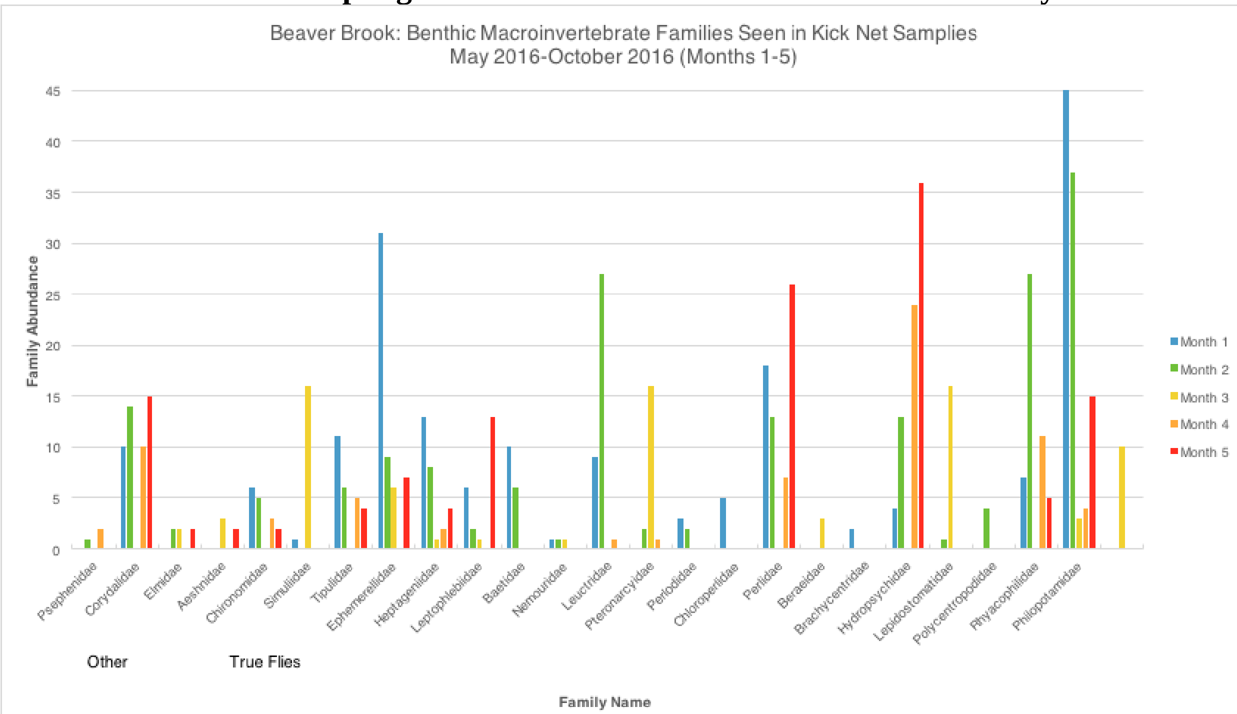
Mazzone, M. , A. Villamagna, B. O'Donnell. 2017. Assessing Salt Stress on Macroinvertebrate Communities in NH Streams. Plymouth State University Showcase of Excellence (oral presentation)

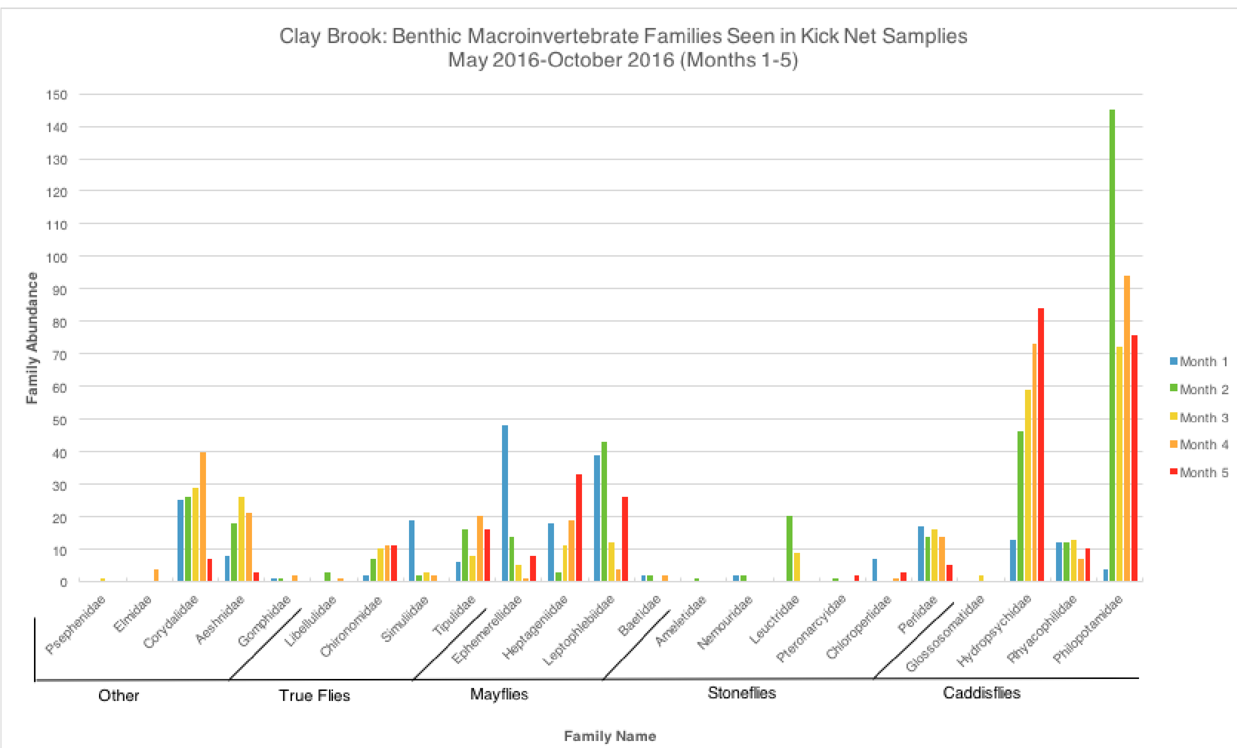
Number of students supported*:* 2 master's students, Matthew Mazzone and Roy Fruit. 2 undergraduate students, Ryan Duquette and Thomas Lafortune, were affiliated with the project through university match and research collaboration., but did not receive direct funding from NH WRRC.

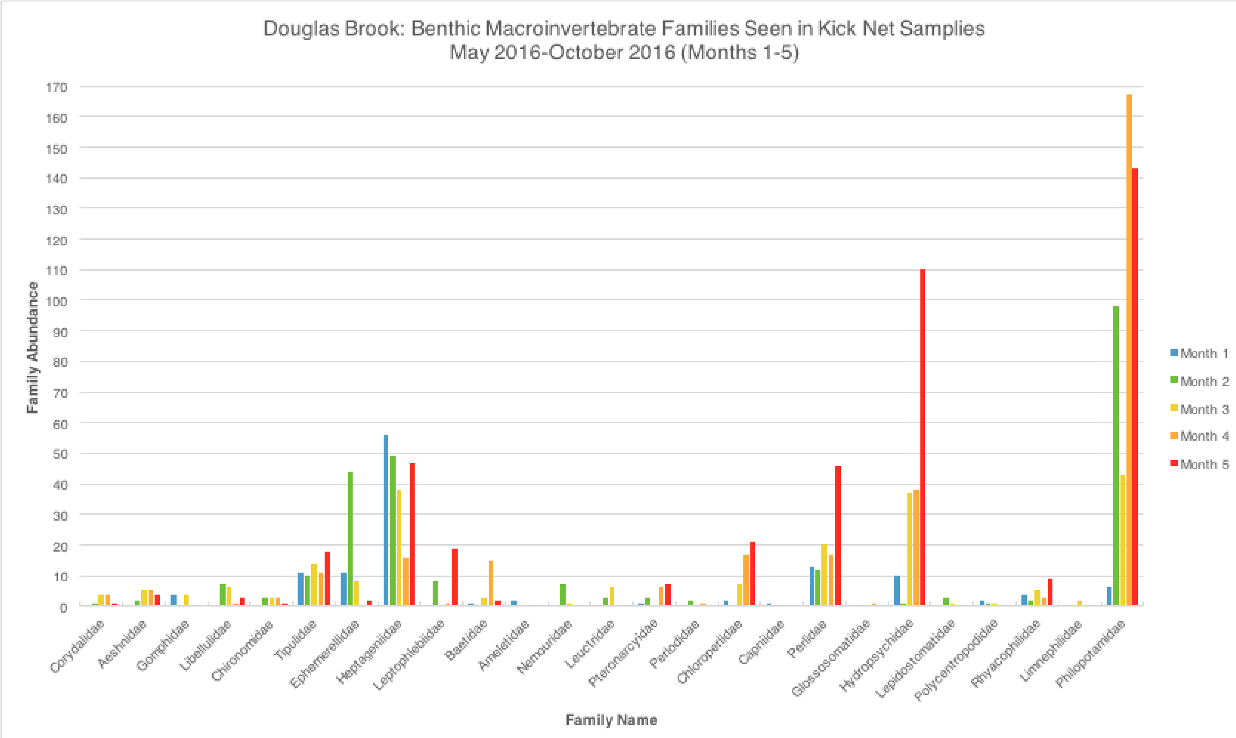
Number of faculty supported: Assistant professor, Amy Villamagna (Ph.D.) received direct funding for this project and Associate professor, Brigid O'Donnell (Ph.D.) was affiliated with the project through university match and research collaboration.

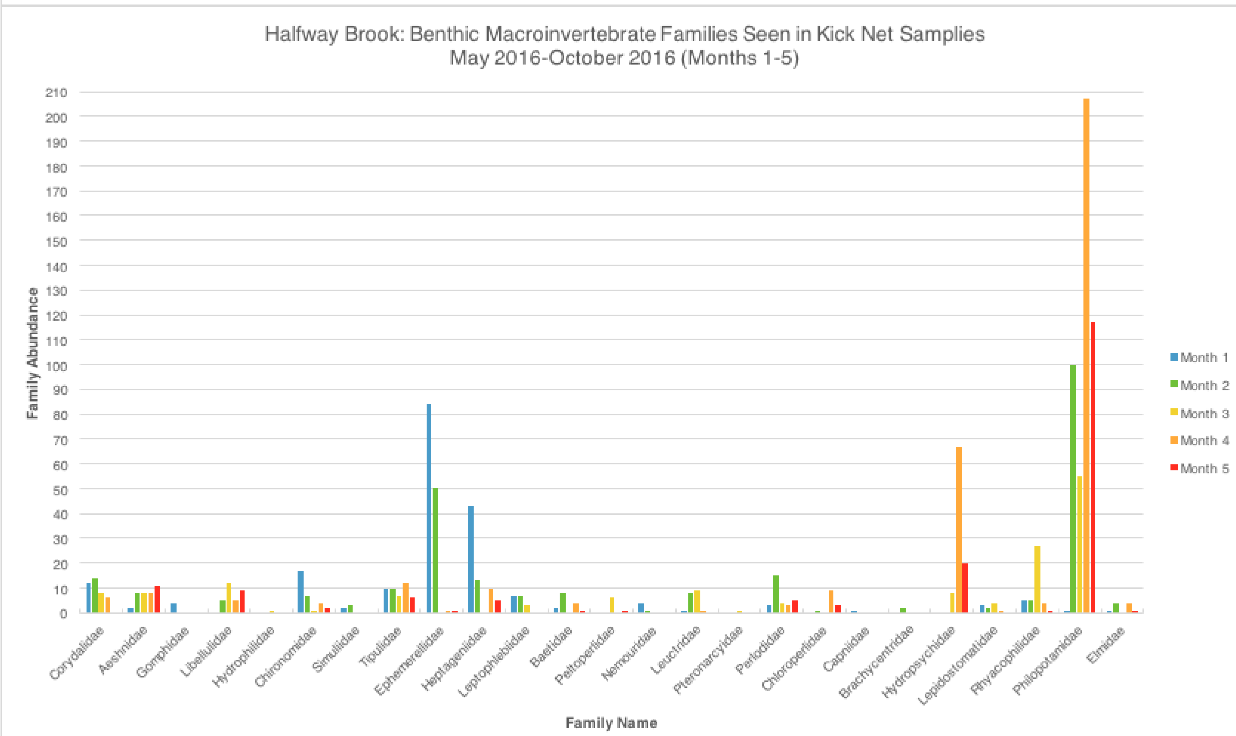
APPENDICES

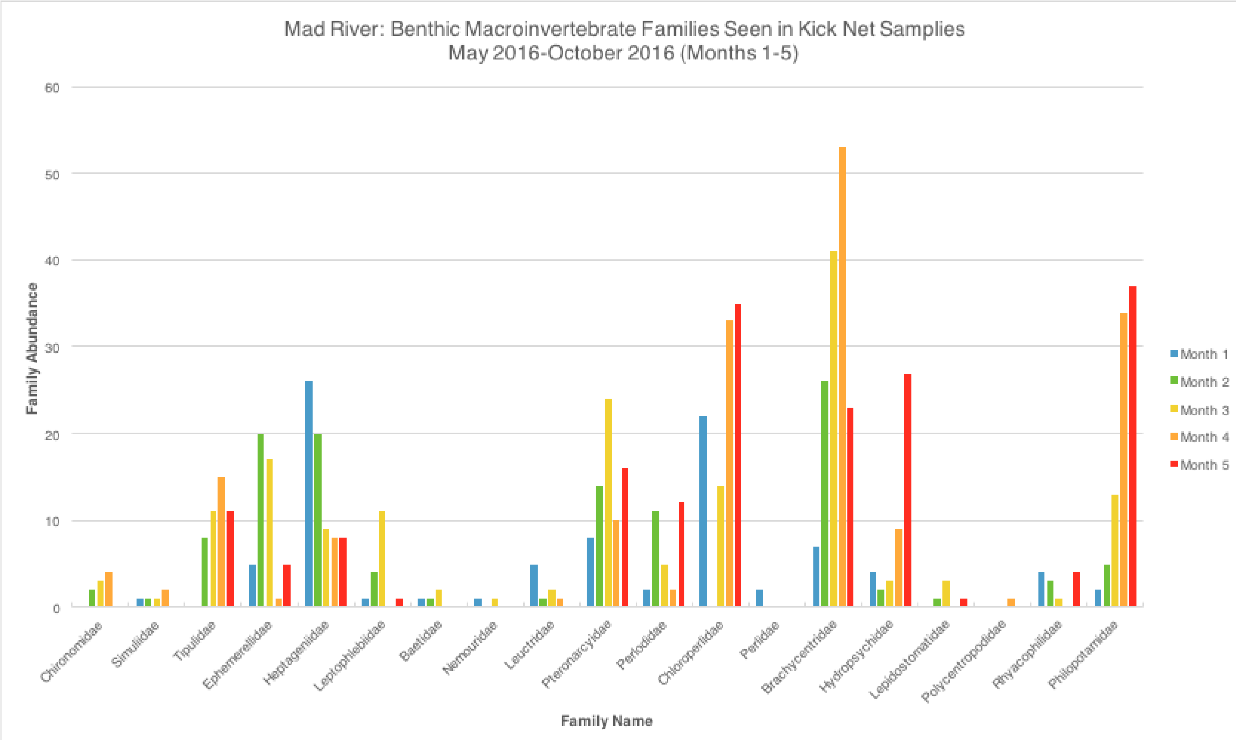
Appendix A: Within season comparison of family level abundance observed through kicknet sampling for benthic macroinvertebrates at all ten study sites.

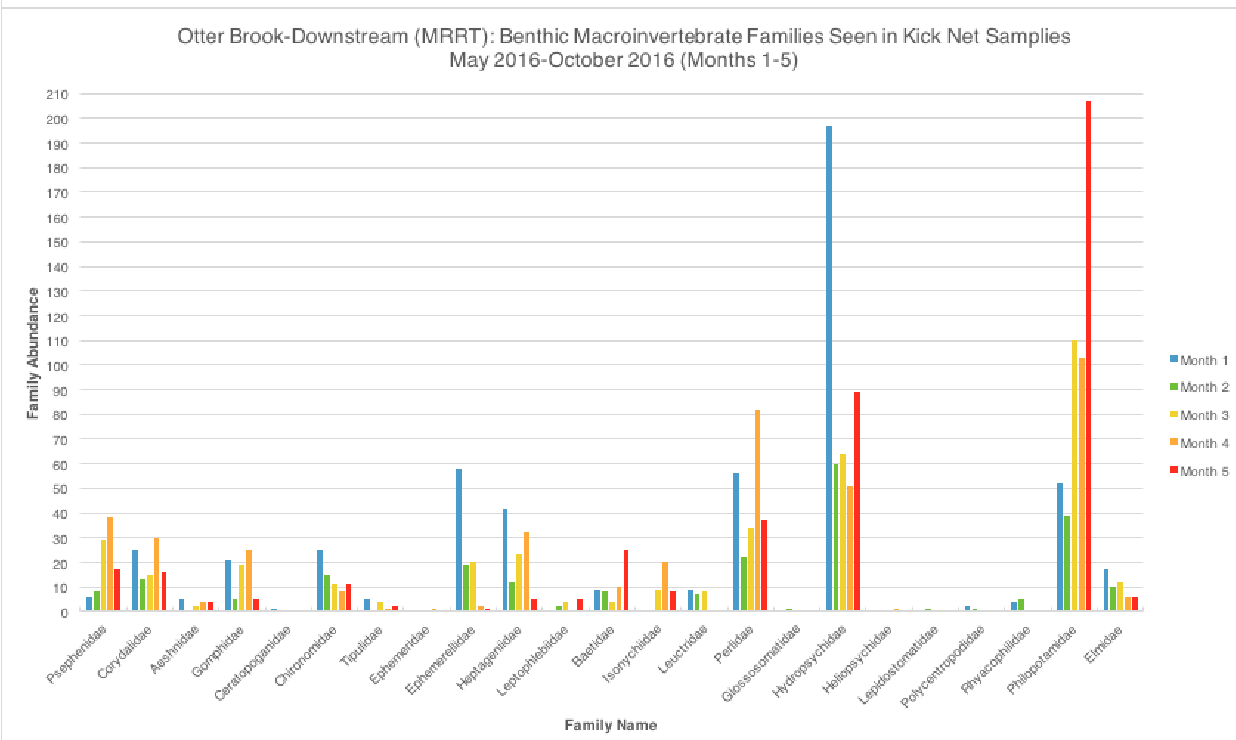


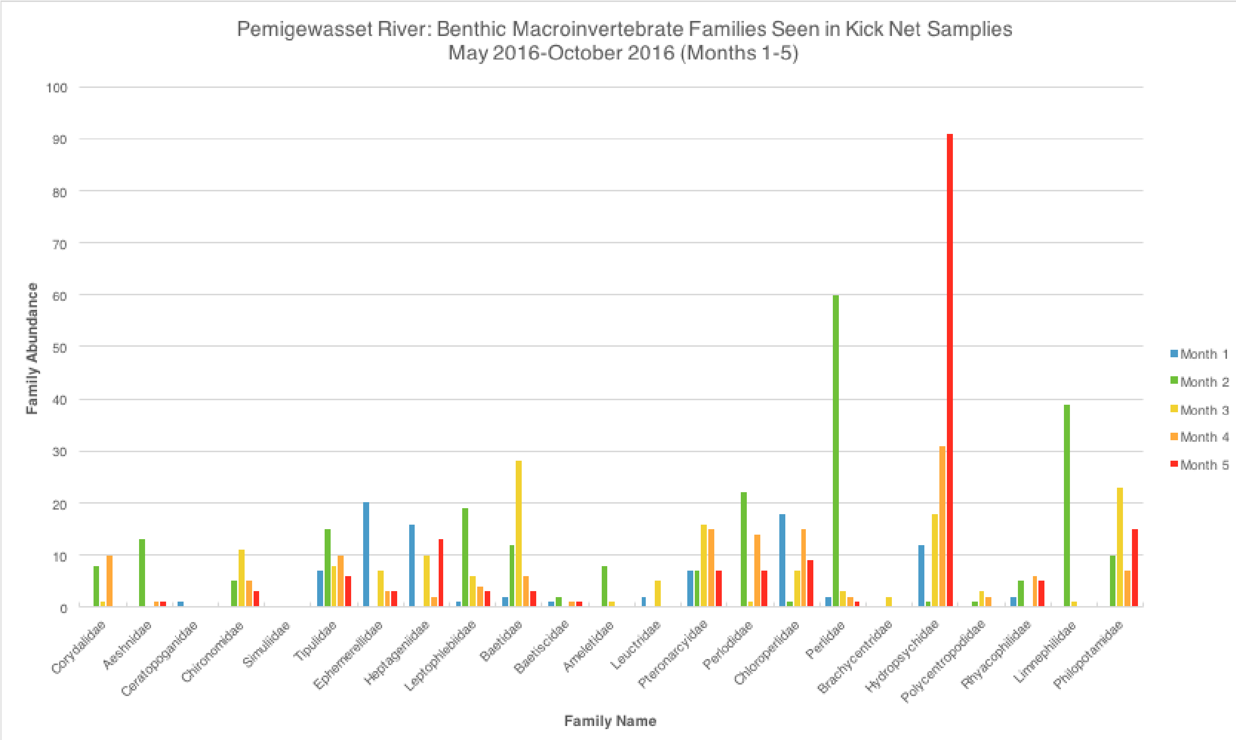


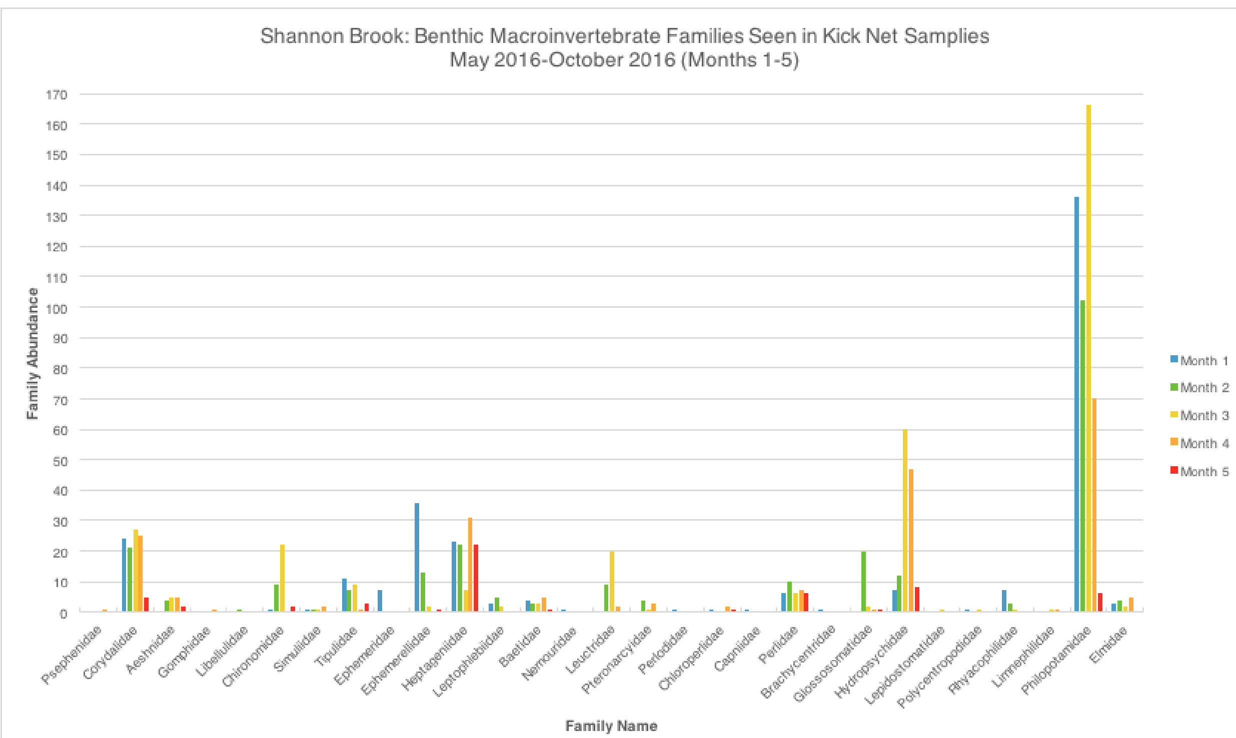

Douglas Brook: Benthic Macroinvertebrate Families Seen in Kick Net Samples
May 2016-October 2016 (Months 1-5)
Family Abundance
Family Name
Month 1
Month 2
Month 3
Month 4
Month 5
Corydalidae
Aeshnidae
Gomphidae
Libellulidae
Chironomidae
Tipulidae
Ephemerellidae
Heptageniidae
Leptophlebiidae
Baetidae
Ameletidae
Nemouridae
Leuctridae
Pteronarcyidae
Perlodidae
Chloroperlidae
Capniidae
Perlidae
Glossosomatidae
Hydropsychidae
Lepidostomatidae
Polycentropodidae
Rhyacophilidae
Limnephilidae
Philopotamidae

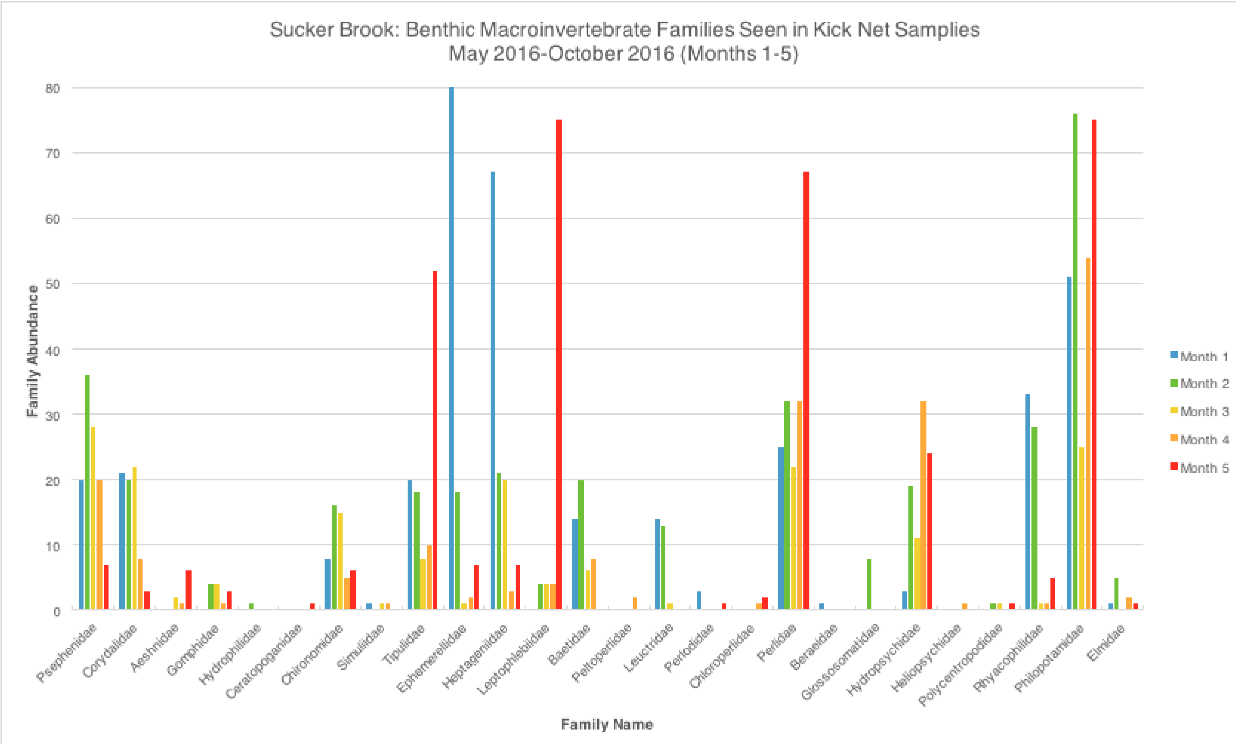

Halfway Brook: Benthic Macroinvertebrate Families Seen in Kick Net Samples
May 2016-October 2016 (Months 1-5)
Family Abundance
Family Name
Month 1
Month 2
Month 3
Month 4
Month 5
Corydalidae
Aeshnidae
Gomphidae
Libellulidae
Hydrophilidae
Chironomidae
Simuliidae
Tipulidae
Ephemerellidae
Heptageniidae
Leptophlebiidae
Baetidae
Peltoperlidae
Nemouridae
Leuctridae
Pteronarcyidae
Perlodidae
Chloroperlidae
Capniidae
Brachycentridae
Hydropsychidae
Lepidostomatidae
Rhyacophilidae
Philopotamidae
Elmidae


Mad River: Benthic Macroinvertebrate Families Seen in Kick Net Samples
May 2016-October 2016 (Months 1-5)
Family Abundance
0
10
20
30
40
50
60
Chironomidae
Simuliidae
Tipulidae
Ephemerellidae
Heptageniidae
Leptophlebiidae
Baetidae
Nemouridae
Leuctridae
Pteronarcyidae
Perlodidae
Chloroperlidae
Perlidae
Brachycentridae
Hydropsychidae
Lepidostomatidae
Polycentropodidae
Rhyacophilidae
Philopotamidae
Family Name
Month 1
Month 2
Month 3
Month 4
Month 5

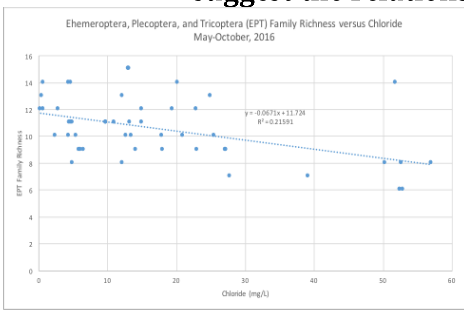

Otter Brook-Downstream (MRRT): Benthic Macroinvertebrate Families Seen in Kick Net Samples
May 2016-October 2016 (Months 1-5)
Family Abundance
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
Psephenidae
Corydalidae
Aeshnidae
Gomphidae
Ceratopoganidae
Chironomidae
Tipulidae
Ephemeridae
Ephemerellidae
Heptageniidae
Leptophlebiidae
Baetidae
Isonychiidae
Leuctridae
Perlidae
Glossosomatidae
Hydropsychidae
Heliopsychidae
Lepidostomatidae
Polycentropodidae
Rhyacophilidae
Philopotamidae
Elmidae
Family Name
Month 1
Month 2
Month 3
Month 4
Month 5


Pemigewasset River: Benthic Macroinvertebrate Families Seen in Kick Net Samples
May 2016-October 2016 (Months 1-5)
Family Abundance
Family Name
Month 1
Month 2
Month 3
Month 4
Month 5
Corydalidae
Aeshnidae
Ceratopoganidae
Chironomidae
Simuliidae
Tipulidae
Ephemerellidae
Heptageniidae
Leptophlebiidae
Baetidae
Baetiscidae
Ameletidae
Leuctridae
Pteronarcyidae
Perlodidae
Chloroperlidae
Perlidae
Brachycentridae
Hydropsychidae
Polycentropodidae
Rhyacophilidae
Limnephilidae
Philopotamidae

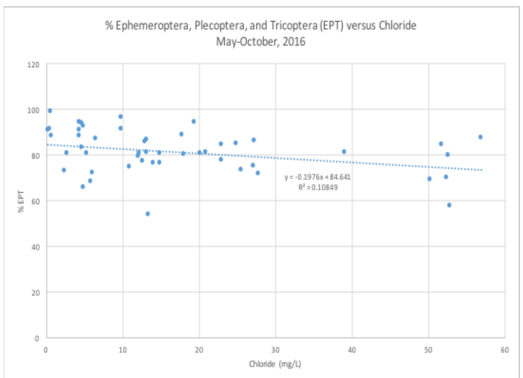

Shannon Brook: Benthic Macroinvertebrate Families Seen in Kick Net Samples
May 2016-October 2016 (Months 1-5)
Family Abundance
Family Name
Month 1
Month 2
Month 3
Month 4
Month 5
Psephenidae
Corydalidae
Aeshnidae
Gomphidae
Libellulidae
Chironomidae
Simuliidae
Tipulidae
Ephemeridae
Ephemerellidae
Heptageniidae
Leptophlebiidae
Baetidae
Nemouridae
Leuctridae
Pteronarcyidae
Perlodidae
Chloroperlidae
Capniidae
Perlidae
Brachycentridae
Glossosomatidae
Hydropsychidae
Lepidostomatidae
Polycentropodidae
Rhyacophilidae
Limnephilidae
Philopotamidae
Elmidae


Sucker Brook: Benthic Macroinvertebrate Families Seen in Kick Net Samples
May 2016-October 2016 (Months 1-5)
Family Abundance
Family Name
Month 1
Month 2
Month 3
Month 4
Month 5
Psephenidae
Corydalidae
Aeshnidae
Gomphidae
Hydrophilidae
Ceratopoganidae
Chironomidae
Simuliidae
Tipulidae
Ephemerellidae
Heptagenildae
Leptophlebiidae
Baetidae
Peltoperlidae
Leuctridae
Perlodidae
Chloroperlidae
Perlidae
Beraeidae
Glossosomatidae
Hydropsychidae
Heliopsychidae
Polycentropodidae
Rhyacophilidae
Philopotamidae
Elmidae

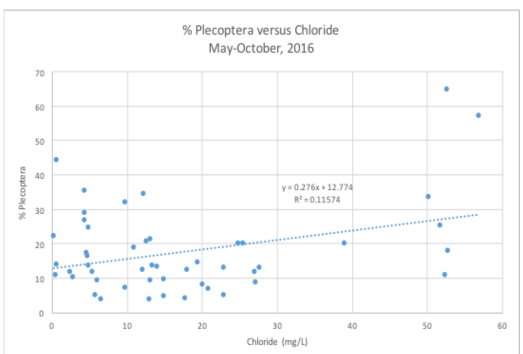
Appendix B: Univariate relationships between chloride and common benthic macroinvertebrate biomonitoring metrics. The graph illustrates the observed relationship across all sampling months in 2016. Yellow highlighted boxes in table suggest the relationship was significant and as hypothesized.



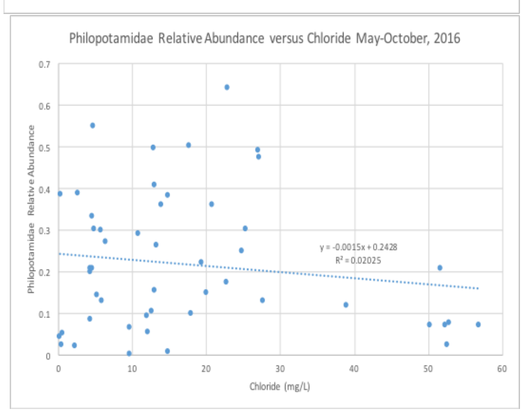
Month	Equation	R^2
May-June	y = -0.0603x + 12.183	0.21106
June-July	y = -0.0221x + 12.08	0.07356
July-August	y = -0.0733x + 12.158	0.13203
August-September	y = -0.099x + 11.506	0.52586
September-October	y = -0.0878x + 10.971	0.55617



Month	Equation	R^2
May-June	y = -0.0009x + 84.463	3.90E-06
June-July	y = -0.1335x + 87.1	0.17894
July-August	y = -0.1632x + 77.697	0.04555
August-September	y = -0.292x + 84.044	0.24641
September-October	y = -0.5147x + 93.763	0.5565



Month	Equation	R^2
May-June	y = 0.5558x + 12.819	0.28156
June-July	y = 0.6511x + 6.6956	0.53375
July-August	y = 0.1862x + 14.897	0.09404
August-September	y = -0.1573x + 18.015	0.05514
September-October	y = -0.0471x + 16.253	0.00675



Month	Equation	R^2
May-June	y = 0.0009x + 0.1004	0.0105
June-July	y = -0.003x + 0.2854	0.14317
July-August	y = -0.0005x + 0.226	0.00242
August-September	y = -0.005x + 0.3646	0.19266
September-October	y = -0.0018x + 0.2873	0.02316

Appendix C: Preliminary results from HSP70 lab trials.

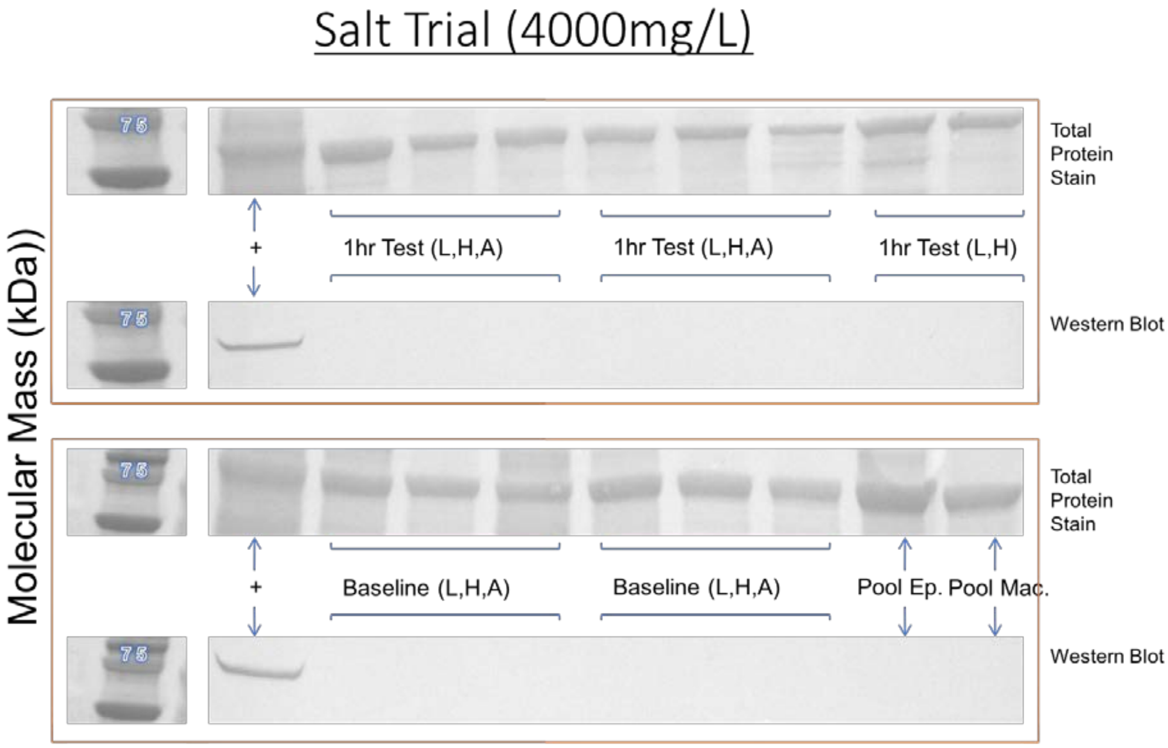


Figure above: Preliminary results from HSP70 lab analysis of mayflies exposed to an acute dose of 4000 mg/L of sodium chloride. The top blot captures the total protein in the sample, and the lower blot in each panel reflects the HSP70. The lane on the far left reflects the positive control for each blot.

Salt & Temperature + Increased P.I.

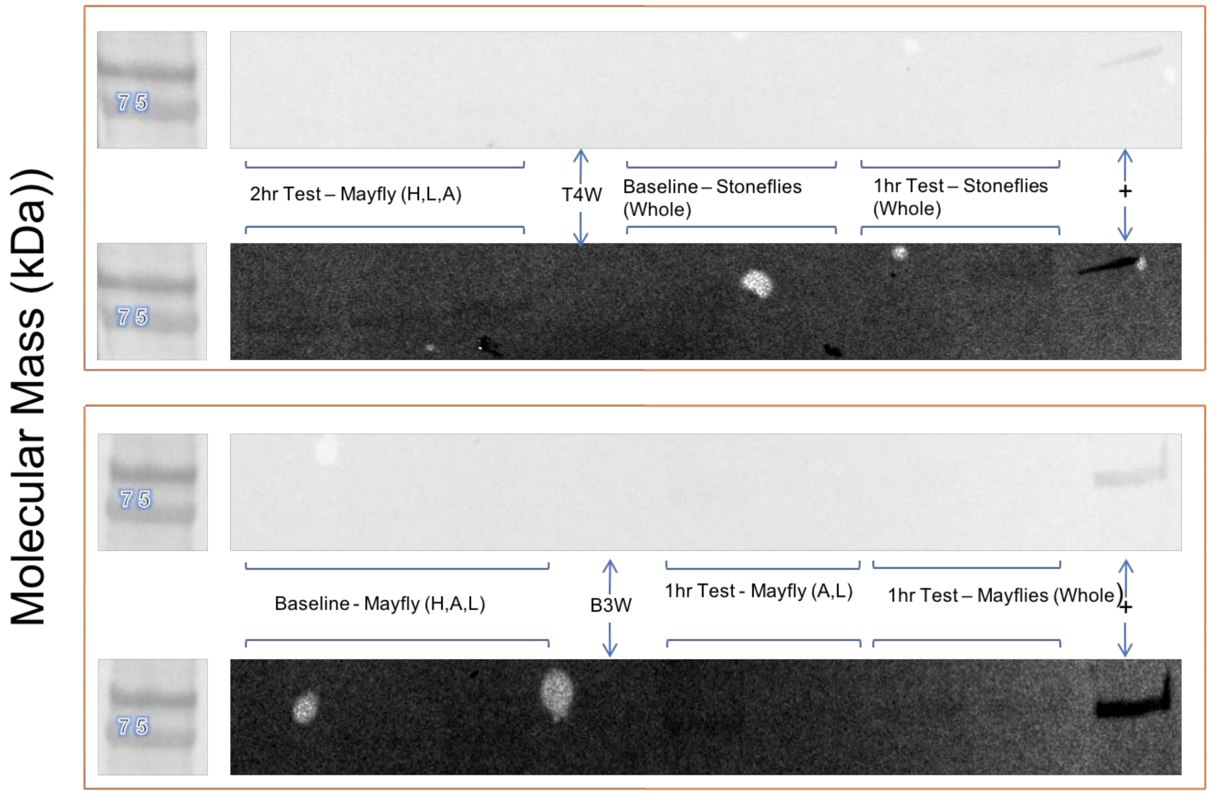


Figure above: Preliminary results from HSP70 lab analysis of mayflies and stoneflies exposed to an acute dose of (potential) salt and temperature stress. The top blot captures the total protein in the sample, and the lower blot in each panel reflects the HSP70. The lane on the far right reflects the positive control for each blot.

Ecosystem Indicators for Freshwater Streams

Basic Information

Title:	Ecosystem Indicators for Freshwater Streams
Project Number:	2016NH202B
Start Date:	3/1/2016
End Date:	2/28/2018
Funding Source:	104B
Congressional District:	NH-1
Research Category:	Water Quality
Focus Categories:	Water Quality, Management and Planning, Non Point Pollution
Descriptors:	None
Principal Investigators:	Alison Watts

Publications

There are no publications.

Improved Ecosystem Indicator Tools for Water Quality Management - NH WRRC Annual Report – Alison Watts, University of New Hampshire

Problem
Water resource managers, such as state and federal agencies, municipalities, and watershed groups, must identify and manage multiple interconnected stressors within an individual watershed. Primary stressors include nutrient inputs, invasive species, water clarity, low dissolved oxygen, contaminants in water and sediment, increased impervious cover, and loss of aquatic buffers and wetlands. Many of these are inter-related, and may be temporally and spatially variable. Ideally, assessment of biotic condition will provide information that allows managers to identify loss of ecosystem function, indicate the relative importance of primary stressors, identify measures or methods to reduce the stress and repair the system, and ultimately track progress towards management goals. Misidentification of stressors may lead to expenditure of management resources without benefit, and contribute to further degradation of the system. Current approaches to assessing the biological integrity of surface waters rely on manual identification of individual species of fish, invertebrates or other organisms. While effective, this approach is labor-intensive and expensive. Furthermore, it assumes *a priori* knowledge of which groups of aquatic biota are most likely to be impacted by water quality; these are the target groups for which identification of individual specimens are obtained. Advances in DNA methods and rapid reductions in analytical costs present an opportunity to harness this new technology and fundamentally improve our capacity to monitor biological communities and individual species (Bista et al., 2017; Thomsen and Willerslev, 2015). Environmental DNA (eDNA), or DNA present in an environmental sample, includes whole microorganisms (microalgae, bacteria etc.) and fragments of tissue, reproductive and waste products, and other cellular material.

Objectives
This study has two major objectives: (1) Pilot a sampling program to develop statistical correlations between causal parameters (including nutrients, land use and chloride) and microbiotic species attributes for wadeable streams in New Hampshire; (2) to assess the value of genomic analyses of eDNA as an additional tool to evaluate the ecological health of streams.

Methods
The study was conducted at wadeable streams across New Hampshire and Maine, representing a range of land use and stream characteristics. Samples were collected at existing NH Department of Environmental Services (NHDES) Volunteer River Assessment Program (VRAP) and Long Term Monitoring sites, in coordination with NHDES staff and volunteers. The NHDES VRAP program engages over 150 volunteers to sample 30 sites, and provides data that contributes to stream assessment associated with more than 2,900 miles of rivers and streams in New Hampshire. Volunteers have been trained to collect samples for genomic analysis, and the methods, results, and implications will be shared with volunteers and watershed groups. Water samples collected from these sites in 2016 and 2017 were analyzed by amplicon sequencing to provide data on stress response, seasonality,

replicability, and trends. Additional samples collected from Maine included attached algae (periphyton) samples collected by the Maine Department of Environmental Services (MEDEP).

Sampling was conducted from June – October 2016,at approximately 30 sites, and 10 sites were re-sampled monthly in 2017. Samples were conveyed to UNH and frozen pending extraction and sequencing of DNA. Most of these samples have been sequenced to identify bacteria, while a smaller subset have been sequenced for eukaryote animal and plant species. Initial analysis and taxonomic identification was performed via QIIME2 (Quantitative Insights Into Microbial Ecology; Caporaso et al, 2010) with the Genbank database.

Principal findings and significance

We identified approximately 40,000 named bacterial species, and 300 animal species (primarily representing fragments, not whole organisms) in the sample set. Analysis of microbial diversity indicates strong correlation with water quality parameters (Figure 1). Specifically, alpha microbial phylogenetic diversity decreases as certain stressors dominate; decreasing diversity was measured in response to increasing temperature, decreasing dissolved oxygen (which are correlated), and increasing nutrients (nitrogen and phosphorus).

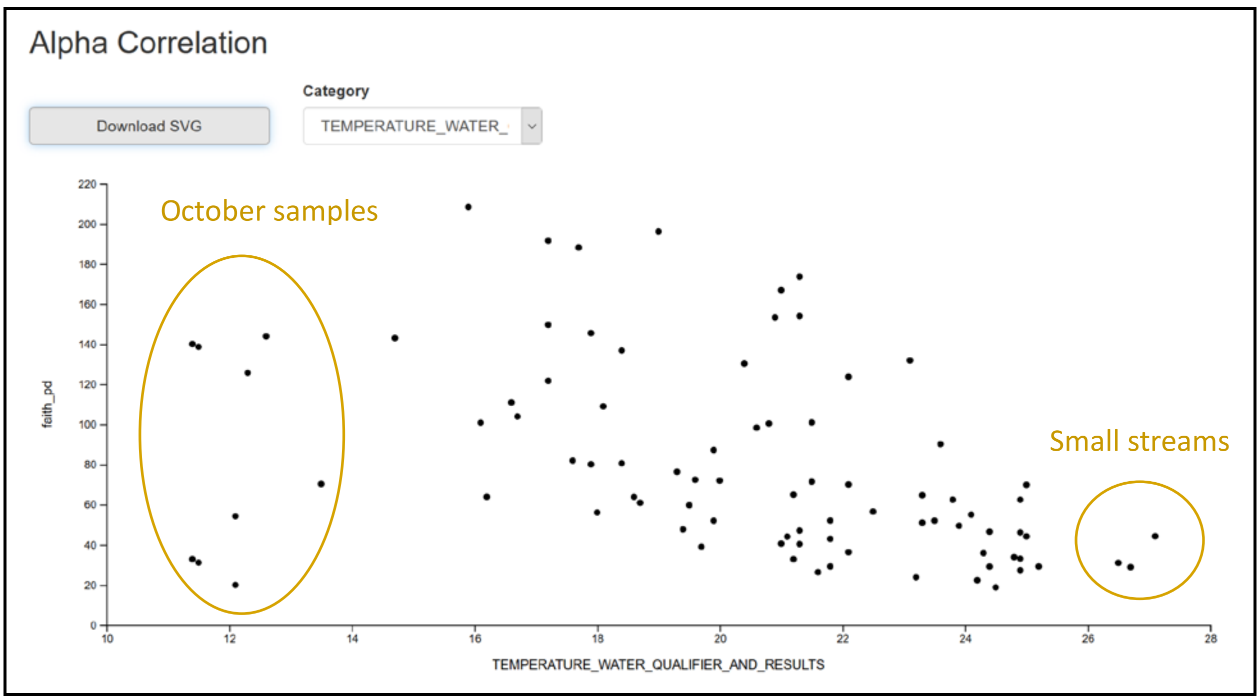


Figure 1. Microbial phylogenic diversity (pd) declines in response to increasing water temperature in NH streams. Samples collected in October show generally higher diversity, indicating that microbial community recovers fairly quickly in the fall when temperatures decrease. A similar trend of decreasing diversity with stressors is seen for nutrients (phosphorus and nitrogen) and dissolved oxygen.

Samples were also collected from stream periphyton (algae) in 2017. Periphyton is used to support stream classification in several states including Maine. Samples were collected from streams in Maine by scraping algae directly from rocks, and in New Hampshire using glass slides as a substrate. Samples were extracted and amplified with an 18S primer to identify eukaryote species. The data set is limited to 20 samples, but shows a potential correlation between stream classification and periphyton species (Figure 2). Samples collected in New Hampshire are distinctly different from samples collected in Maine, which we attribute to the difference in collection methods. The DNA method provides detailed information on number of species, and community composition, but no information on species counts.

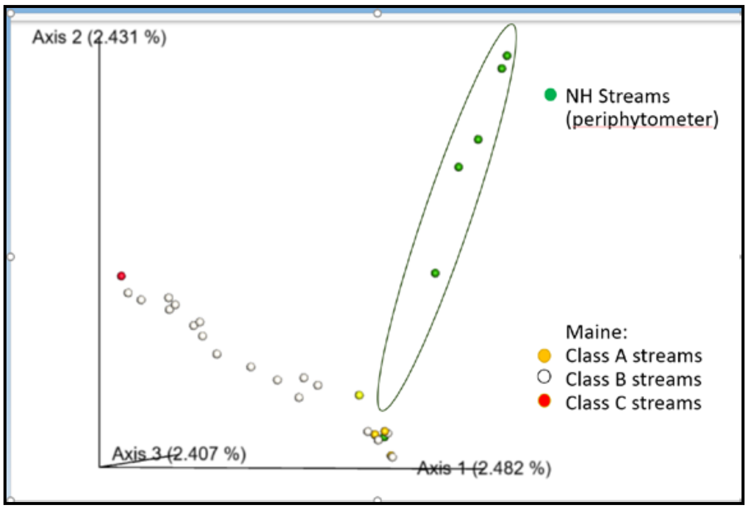


Figure 2. Principal components analysis of algae communities in small streams. Samples which are grouped together have similar algae communities. This preliminary data suggests that amplicon DNA analysis can support stream classification.

These results indicate that DNA-derived periphyton community analysis may be useful for developing or monitoring stream clarification and condition indicators.

Publications and presentations

Presentation: *Metabarcoding and aquatic bacteria in streams: What can microbiology tell us about the big picture?* A. Watts, T. Walsh, T. Danielson. Society of Toxicology and Chemistry, Minneapolis, MN. Nov 2017.

New technology and New Issues in Watershed Management – eDNA Methods for NH Streams. A. Watts. Lamprey River Symposium. Durham, NH, Jan 2018

Outreach or Information Transferred

We have engaged volunteer organizations in sampling collection in NH streams. Outreach materials and fact sheets have been prepared for volunteers (Figure 3).

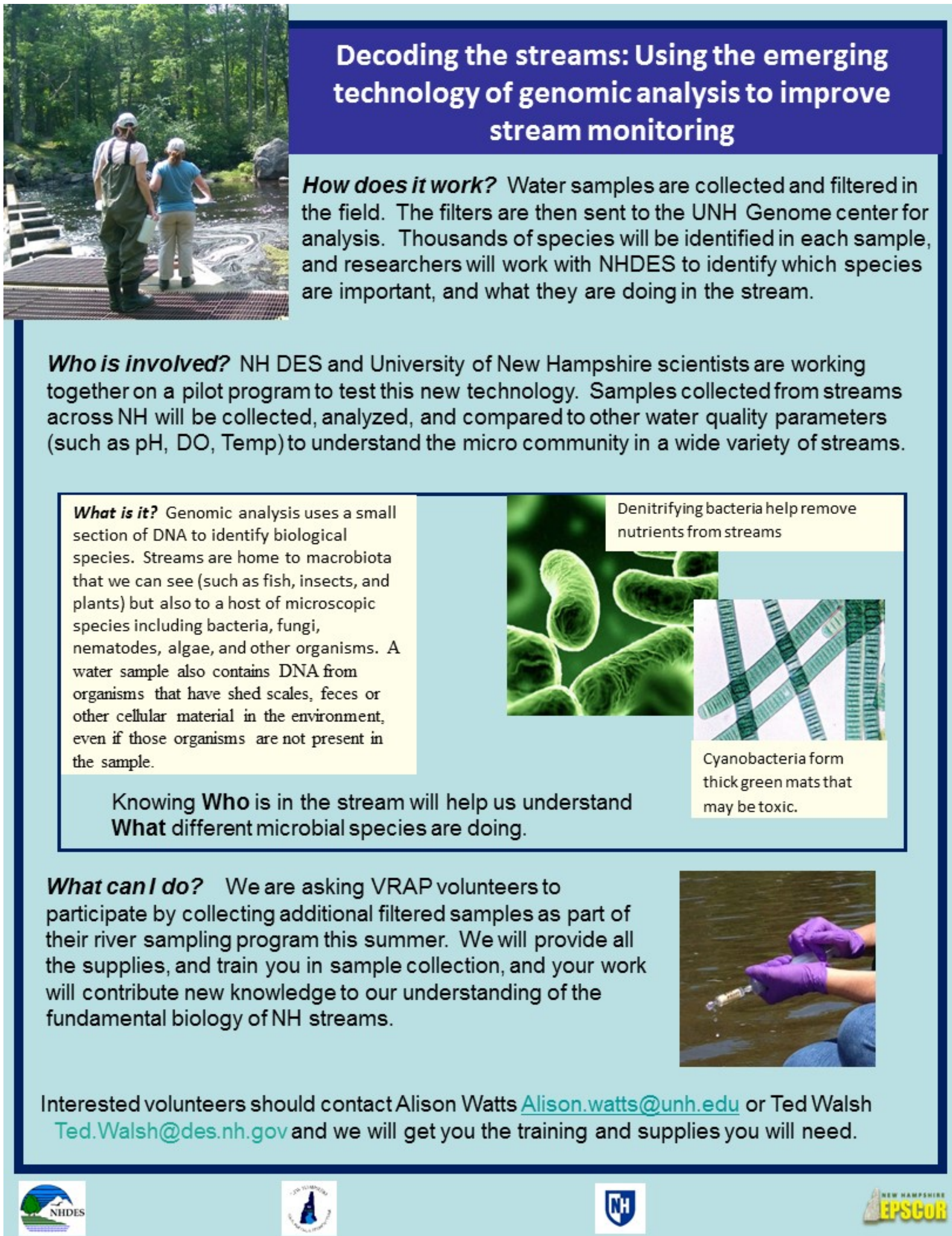


Figure 3. Fact sheet distributed to VRAP volunteers.

Number of students supported

This project has provided partial support for three undergraduate students (Kendra Dow, Tim Sommers, and Cassidy Wallich, all seniors in Environmental and Civil Engineering).

Number and names of faculty and staff supported

Alison Watts, Research assistant Professor

Effects of dissolved organic carbon on methylmercury bioavailability in stream ecosystems

Basic Information

Title:	Effects of dissolved organic carbon on methylmercury bioavailability in stream ecosystems
Project Number:	2016NH205G
USGS Grant Number:	
Start Date:	9/1/2016
End Date:	8/31/2018
Funding Source:	104G
Congressional District:	2nd Congressional district of New Hampshire
Research Category:	Water Quality
Focus Categories:	Surface Water, Geochemical Processes, Toxic Substances
Descriptors:	None
Principal Investigators:	Kathryn L Cottingham, Celia Y. Chen, James Shanley

Publications

There are no publications.

Effects of dissolved organic carbon on methylmercury bioavailability in stream ecosystems (2016NH205G)

Problem: Neurotoxic methylmercury bioaccumulates through aquatic food webs and is a primary cause for fish consumption advisories in the Northeast. The mobilization, transport and bioavailability of mercury in aquatic ecosystems is strongly tied to organic matter dissolved in the water, yet levels of methylmercury in watersheds, and that bioaccumulate in fish, are difficult to predict. Previous studies have noted that relationships between stream methylmercury and dissolved organic carbon (DOC) in streams changes over time. There is also a seemingly contradictory effect of DOC on uptake by the biota: at low concentrations of organic carbon, methylmercury bioaccumulation increases, whereas at higher concentrations, uptake into fish and invertebrates is attenuated. This project is testing the hypothesis that differences in the chemical structure of the DOC that is transporting MeHg in streams lead to the temporal changes and non-linearity in bioaccumulation noted in previous studies.

Objectives:

1) Identify and characterize fractions of DOC that associate with MeHg and Hg in streams,
2) Determine the effects of DOC quality and quantity on MeHg uptake by primary producers at the base of the stream food web.

Methods:

Field study: A spatial and temporal study of dissolved organic carbon quantity and quality, and water MeHg and Hg levels in the tributaries of Lake Sunapee was completed in the summer of 2017. Water samples were collected in April and August from 27 sites, as well as from three contrasting sites on a bi-weekly basis. Filtered water samples from field collection were analyzed by 3D excitation-emission fluorescence spectroscopy, and by ultraviolet spectroscopy within 48 h of collection. Whole and filtered water samples were also analyzed for total organic carbon, MeHg and Hg. Fluorescence excitation-emission matrix (EEM) data was analyzed in Matlab using the "Dr. EEM" toolbox, and corrected for instrument and inner filter effects. Components of organic carbon were identified from *a priori* fluorescence and UV indices, and PARAFAC analyses was performed on EEM matrices to identify organic carbon components specific to this watershed.

Our USGS collaborators collected samples from a reference watershed in Sleepers River, VT, to provide some comparison to the current watershed. Samples were analyzed for MeHg, Hg at Dartmouth, and for organic carbon quality and concentration by our USGS collaborators. Organic carbon quality data and MeHg/Hg concentrations from two other watersheds, collected during a previous study, were also provided by our USGS collaborators.

In August 2017, biofilm and invertebrate samples were collected from 11 streams at sites near the lake. Biofilm was analyzed for ash content, chlorophyll-a, $\delta^{34}S$. Biofilm and invertebrates were analyzed for MeHg, Hg, $\delta^{13}C$ and $\delta^{15}N$.

Biofilm growth: Our earlier attempts at biofilm growth gave us experience with managing nutrient regimes, but extremely heterogenous spatial distribution of biofilm thickness and algal taxa developed due to uneven flows in the cattle tanks that were originally adapted as artificial streams. We have constructed 4 artificial streams (2m x 0.5m) with paddles for water circulation, providing a more laminar flow to improve the homogeneity of biofilm growth. These streams will be used to complete the research on Hg uptake in biofilm under controlled conditions.

Mapping: Watershed landscape features for the Lake Sunapee watershed and three reference watersheds were assessed along stream buffer zones using high resolution aerial imagery and high-resolution lidar elevation models in ArcGIS.

Principal findings and significance: Modeling and statistical analysis of the fieldwork data from year 1 is in progress. Much higher levels of MeHg and Hg were present in the Lake Sunapee watershed than the reference watershed sites. Concentrations of MeHg were higher in the late summer than in the spring, and correlated spatially with %wetland in the watershed. Higher concentrations of both humic and protein-like organic matter occurred in streams that passed through wetlands, whereas spectral slope (S_R), a proxy for molecular weight, was correlated with streams with open water (small lakes) upstream in the catchment. Concentrations of MeHg in the water column were weakly inversely correlated with S_R, and with the ratio of protein-like (microbially-mediated) to humic-like organic carbon fractions.

Methylmercury in biofilm was not correlated to MeHg in stream water, likely because of the variable composition of biofilm between streams, whereas %MeHg in biofilm was inversely related to %ash (inorganic material). Methylmercury in grazing invertebrates (caddisfly, mayfly) were correlated with MeHg and DOC in the water column at lower concentrations, but sites with the highest concentrations of DOC had lower uptake of MeHg in the biota.

Our preliminary findings suggest sites associated with wetlands export higher levels of MeHg, but bioavailability of MeHg associated with organic carbon from wetlands is lower than from streams without wetlands. The higher ratios of protein-like to humic-like organic matter originating from wetlands may reduce the availability of MeHg for uptake to the base of the foodweb. This hypothesis is being further tested under controlled mesocosm conditions using artificial streams.

Publications and presentations: Findings from the first year of research will be presented at the American Society of Limnology and Oceanography annual meeting in June 2018.

Notable awards and achievements: Co-PI Celia Chen and investigator Vivien Taylor received a CompX grant through the Neukom Institute at Dartmouth to compare findings from Lake Sunapee with other watersheds. The CompX grant has enabled us to improve our GIS and modeling capabilities.

Investigator Vivien Taylor received an NSF grant to study sources of methylmercury to fish in Lake Sunapee. This work will build on the current grant by comparing the isotopic ratios of mercury in the tributaries and in the lake, to determine whether bioavailable mercury is transferred from the watershed to the lake, or produced from in-lake processes.

Outreach and information transferred: We are in contact with the Lake Sunapee Protective Association and met with them later in May 2017 to talk with them about our prior studies on Lake Sunapee as well as the current research. In August 2017, three students associated with the project attended the Lake Sunapee Bass Derby and provided information to local fisherman about mercury in fish.

Students supported: (2)

Keith Kantack (Master’s student), worked full time on the project for 2.5 months.

Paul Vickers (2nd year undergraduate), worked full-time on the project spring term, and part-time in the winter and summer terms.

Faculty and staff supported: (5)

Kathy Cottingham (Professor)

Celia Chen (Research Professor)

Vivien Taylor (Research Scientist)

Kate Buckman (Research Scientist)

Seth Roberts (Research Assistant)

Special story and photo:

"Being a part of the Dartmouth WRRC team was a great opportunity to apply some of my GIS and watershed science skills, but also to learn hands on about aquatic ecology and environmental chemistry. My experience on the team made me a stronger applicant for the job I now have with a river restoration firm, where I work to improve riparian habitat in the New England and the Pacific Northwest." Keith Kantack, Dartmouth MSc Earth Science 2017

Keith joined the WRRC research team while finishing up his Master’s degree in Earth Science, and applied his expertise in remote sensing and mapping to analyzing watershed landscape characteristics of Lake Sunapee and three reference watersheds. Keith also expanded his research skills by collecting and processing stream water samples for our field study, and learned to identify and collect biofilm and invertebrate samples for ecological assessment. Following the field season, he led a field laboratory session on stream invertebrates as part of the Dartmouth ‘Stretch’, an off-campus field program for Earth Science majors. Since gaining applied research experience on the WRRC Lake Sunapee project, Keith has started working for a river restoration company in New England, and is advancing his career in watershed research.

Hot and Salty: Assessing ecological stress in New Hampshire streams at community, population, and molecular levels

Basic Information

Title:	Hot and Salty: Assessing ecological stress in New Hampshire streams at community, population, and molecular levels
Project Number:	2017NH210B
Start Date:	3/1/2017
End Date:	2/28/2018
Funding Source:	104B
Congressional District:	NH-02
Research Category:	Water Quality
Focus Categories:	Ecology, Methods, None
Descriptors:	None
Principal Investigators:	Amy Marie Villamagna

Publication

1. Mazzone, M. 2018. ‘The Impacts of Chloride on Macroinvertebrate Communities in New Hampshire Streams’. Master of Science in Environmental Science & Policy. Plymouth State University. Plymouth, NH (USA)

Hot and Salty: Assessing ecological stress in New Hampshire streams at community, population, and molecular levels

Problem

New Hampshire's climate is expected to resemble that of the US Mid-Atlantic by 2100 (USGCRP 2009). With this shift comes increased air temperatures, less snow pack, more ice storms, and more rain on snow events. From a freshwater ecology perspective, much of central and northern New Hampshire's streams are currently populated by coldwater species (e.g., Brook trout; Neils 2009). As a result of increasing air temperatures, stream temperature will likely increase; however, the increase will vary among streams (Kelleher et al. 2011). For many species, this thermal shift may be within their fundamental tolerance range (e.g., 21°C thermal maxima for Brook Trout), barring additional physiological stress. However, growth in development (e.g., roads, housing) and energy production (mining, fracking) in northeastern states is causing additional stress on freshwater biota (Van Meter et al. 2011, Kelting et al. 2012). Among emerging concerns are the short-term and cumulative impacts of thermal and salinity stress on freshwater resources and biota (Findlay and Kelley 2011, Cuffney et al. 2010, Van Meter et al. 2011, Dalinsky et al. 2014, Stitt et al. 2014).

Road deicers are an emerging concern in NH where a 'bare pavement' policy has been adopted (TRB, 1991). Applied salts are flushed from roadways in early spring and during mid-winter thaw events. Some are immediately incorporated into surface waters while others infiltrating into subsurface flow and groundwater before reaching streams (Daley et al. 2009). The movement of sodium chloride into soil and groundwater systems delays the emergence of salts in streams, resulting in elevated Cl concentrations into summer months (Williams et al. 2000, Findlay et al. 2011, Kelting et al. 2012), a sensitive time for growth, development, and reproduction of freshwater biota. The impacts of thermal variability and salt loading on freshwater biota have garnered attention and study in northern states, but it remains unclear how the synergy of salt and thermal stressors impact biota across the community, population and molecular levels.

Traditionally, biotic response to water quality degradation is measured using broad-based community metrics (e.g., Simpson's Index of Diversity) and/or assessing populations of select bio-indicators (e.g., EPT= the macroinvertebrate orders of Ephemeroptera, Plecoptera, and Tricoptera). More recently, researchers look to family and genus level abundance as indicators of water quality (Carlisle et al. 2008). However, both approaches are largely reliant on the loss of individuals and/or species, which could have cascading effects on biodiversity and the ecological function of streams. In order to avoid the potentially negative effects of osmo-thermal stress on NH stream biota, we need studies that investigate subtle biotic responses along a gradient of salt and thermal stress. Collectively, this means monitoring the overall composition of the benthic macroinvertebrate community as well as stress at the individual level via biomarkers.

Biomarkers are parameters serving as objective and quantifiable characteristics of biological processes. These can include indications of unintended environmental exposure (Strimbu & Tavel, 2010). A good biomarker is one that can be used to model dose-effect relationships for clinical diagnoses and monitoring purposes (Van Der Oost et al. 2003). We sought a biomarker capable of representing the sublethal stress response to NaCl. Harmful effects of chemical introduction into the environment may not be readily apparent; some deleterious effects at the organismal level will only be visible after a series of molecular events. Morales et al. (2011) suggested that using subcellular biomarkers of stress is advantageous due to their high sensitivity and fast response to stressors as compared to higher levels of organization (Morales et al. 2011). Sublethal cellular responses to stress can include rapid changes to metabolism, nutrient uptake, cell cycle growth, and the survival time of cells (Kroemer et al. 2010). Several methods have been suggested for quantifying sublethal stress, include the monitoring of reproductive habits and growth rates (Yousef & Courtney, 2003; Petes et al. 2008). Promise also resides in the form of stress protein quantification (Martin, 2000; Petes et al. 2008). Heat shock proteins (HSPs) are a class of molecular chaperones which aid in the protection/refolding of denaturing and aggregating proteins. HSPs are induced from a variety of stresses, including increased salinity, though they were initially discovered in the cells of organisms exposed to high temperatures. (Hochachka & Somero, 1984; Hill et al. 2012). During instances of biotic and abiotic cellular stress HSPs are rapidly upregulated, allowing them to serve as molecular indicators of stress (Lund et al. 2003; Lencioni et al. 2009; Hochachka & Somero, 1984; Zhao & Jones, 2012).

HSP70 has been demonstrated as inducible in the cells of insect larvae, making it a prime choice for our sublethal stress assessment protocol. De Jong et al. (2006) showed that chloride cells in the abdomen and gills of mayfly larvae expressed high levels of HSP70 in individuals impacted by road salt runoff. Stress protein expression across specific tissues and organs varies among species, individuals, and within different tissues of the body; also, the level of observed expression may be dependent on exposure time to a stressor or the time of year (Krebs & Feder, 1997; Singh & Lakhotia, 2000; Hyne & Maher, 2001). By establishing if/where HSP70 expression is concentrated, researchers may be able to interpret how osmotic stress is introduced and amplified in aquatic nymphs, such as by feeding, osmoregulation, or oxygen intake.

After choosing the mechanism by which stress would be assessed, we selected an organism capable of serving as a bioindicator. Bioindicators are organisms or environmental traits capable of serving as reliable indicators of environmental health (Stocker, 1980). Due to their importance in freshwater ecosystems, we chose to use macroinvertebrates. These animals are important to the structure and function of freshwater ecosystems, and their minimization or loss will potentially affect other trophic levels (Benbow & Merritt, 2004). Of the various macroinvertebrate groups, stoneflies (order: Plecoptera) are a favorable choice. This is because of their availability in streams during all seasons, the ease of collecting them, and their large size, which allows for examination of stress at the individual level (Gaufin & Tarzwell, 1952; Kohler et al. 1992).

Objectives

The goal of this project was to enhance biomonitoring efforts and early detection of thermal and salt stress on stream biodiversity in New Hampshire and to develop techniques that will provide an early-warning signal of ecosystems in jeopardy. Our project objectives were to:

1) **Evaluate differences in stream macroinvertebrate communities along a salt stress gradient.** This objective was met by evaluating macroinvertebrate community composition within ten 1st to 4th order wadeable streams across NH that varied along a salt gradient classified using snapshot water chemistry data from 2013, 2014, and 2016 as well as continuous monitoring of stream conductivity between 2013 and 2016.
2) **Evaluate differences in stream macroinvertebrate communities along a thermal gradient.** This objective was met by evaluating macroinvertebrate community composition within ten 1st to 4th order wadeable streams across NH that varied along a thermal gradient classified using 2016 snapshot and continuous monitoring of stream temperature.

3) **Evaluate sub-lethal osmotic stress in stonefly larvae** by quantifying heat shock protein (HSP70) expression in stoneflies (genus *Acuernaria*) using immunoblotting. This objective will be met by first conducting in-lab salt exposure trials using nymphal stoneflied to evaluate HSP expression in response to salt (NaCl) and thermal treatments.

4) **Compare and evaluate benthic macroinvertebrate sampling techniques and potential indicator taxa for salt stress.** The NH Department of Environmental Services (NHDES)– Biomonitoring program has adopted a rock basket approach for assessing water quality using indicator taxa and community metrics. We set out to compare the rock basket approach to kicknetting over the months of July –September/October to evaluate their ability to detect small changes in community composition that may be attributed to elevated salt or temperature.

This project had five main field and components: 1) field sampling of macroinvertebrates to provide community and population (family-level) metrics of ecological response, 2) laboratory based stress experiments to determine HSP induction thresholds for thermal-salt stress in two mayfly species, 3) field sampling and HSP expression assays of mayfly nymphs from streams that span a thermal and salt gradient to determine the utility of HSPs as biomarkers of stress in wild populations of mayflies, 4) continuous monitoring of conductivity, water level, temperature (stream and air), and 5) snapshot water chemistry (anion and cation) sampling to coincide with macroinvertebrate sampling.

Methods

Site selection

Field sites were selected in 2016 by using GIS to overlay the LoVoTECS network of stream monitoring sites with fish sample sites between 2009 and 2015. From this subset of NH streams, we selected sites based on median chloride concentrations derived from snapshot water chemistry data collected in May and July 2013 and July, Sept, Oct 2014. Our ten sites ranged

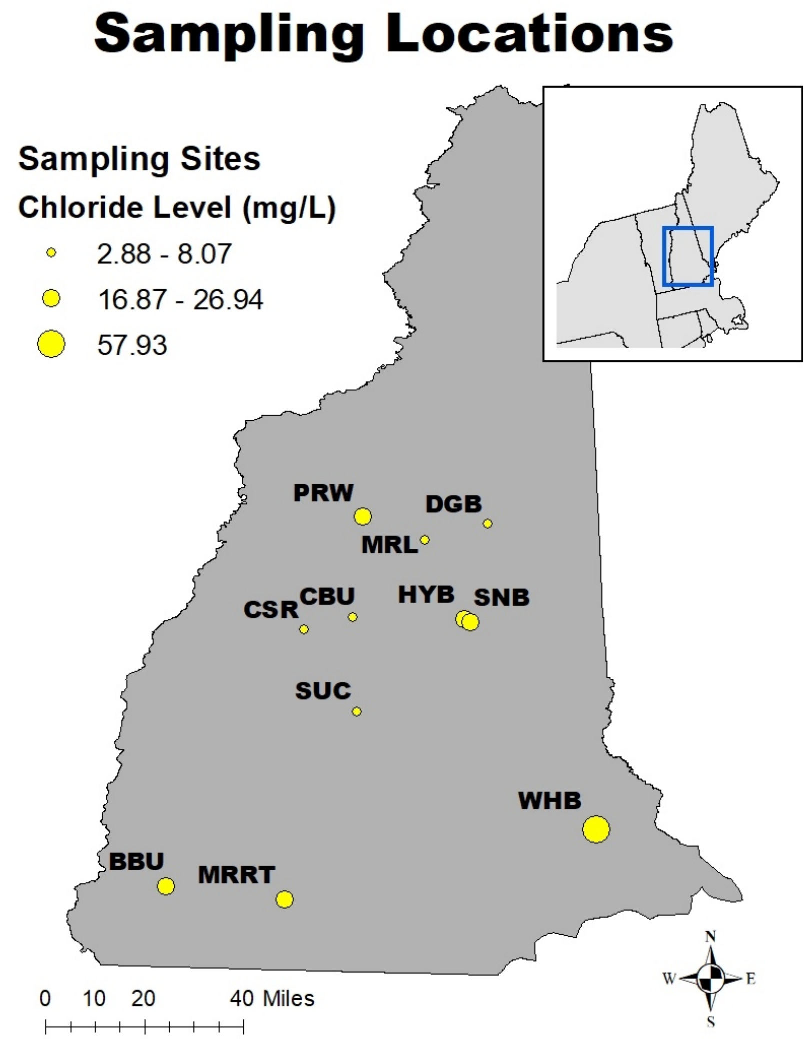


Figure 1:Ten streams sampled for benthic macroinvertebrate and chloride between June and October 2017.

from 4.35-52.6 mg/L of Chloride during this period. The ten sites represent a range of human impact; some sites have roads and development, and some sites have little to no human impact. Two of our original sites, Mad River in Waterville Valley and Douglas Brook near the Kancamangus Highway, are located in the White Mountain National Forest. We noticed a strong correlation between chloride levels at these two sites as well as benthic macroinvertebrate communities. Therefore, we replaced the Douglas Brook site with a new stream reach on the Cockermouth River (Groton, NH) in 2017. The other stream sites are located near minor and major road systems, with minimal to moderate influence from road salts and other anthropogenic influences. The ten sites include: Halfway Brook and Shannon Brook in Moultonborough, Mad River in Waterville Valley, Beaver Brook in Keene, Wednesday Hill Brook in Lee, Pemigewasset River in Woodstock, Clay Brook in Plymouth, Cockermouth River in Groton, Otter Brook in Peterborough, and Sucker Brook in Franklin.

Our research team adopted NAWQA and EPA Rapid Biological Assessment macroinvertebrate sampling protocols for multi-habitat kicknet sampling. We sampled each study stream once every month beginning in late-May to September/October, 2017. At each site, we selected a 100-meter reach that was largely representative of the stream habitat. This 100-m reach was established in close proximity to continuously logging specific conductance, water temperature, and water level sensors; most sites consisted of sample reaches that were 50-meters upstream and 50-meters downstream, or, where that was not feasible, 25-meters and 75-meters. We sampled 10 kicks using a 500 μm net over the 100-meter stream reach, sampling different habitats in approximate proportion to their representation of the total surface area of the reach. We determined this by assigning a percentage of each habitat type (cobble, sand, or large woody debris) totaling 100%. In cobble substrate/habitat, we chose to kick in riffles or runs. In sand substrate and habitat, we mainly kicked in runs and slow moving water since that is the main stream morphology for this type of habitat. We placed all macroinvertebrates in labeled containers with 70% ethanol for preservation. If there were any predator macroinvertebrates, such as the family Corydalidae, then we used an additional container to store the predators.

In addition to kicknetting, we adopted the NHDES biomonitoring program rock basket approach for macroinvertebrate sampling. At each site, we deployed 3 rock baskets side-by-side in a cobble and riffle habitat in close proximity to the continuously logging sensors during the July sampling period. We collected rock baskets roughly after eight weeks in mid to late September to later compare results with NH DES Biomonitoring Program's annual assessments. Our rock basket collection was similar to the NHDES sampling protocol, which included four, 5-gallon buckets, 3 of which will hold the rock baskets themselves, and one bucket to rinse and store the rocks that have been examined. We filled three buckets with stream water a quarter full and facing upstream with the opening facing towards the rock basket. One person lifted each basket into the bucket, making sure to catch any debris that comes loose from the basket. The research team thoroughly examined every rock in each basket, and the water in the bucket was filtered through a 500 μm sieve. We placed all macroinvertebrates in rock basket labeled containers separately to the kick net samples, and stored in 70% ethanol to be preserved. We labeled containers with the correct site name and date sampled. Samples were transported back to Plymouth State University for identification and enumeration.

<u>Macroinvertebrate Identification</u>

Preserved field samples were identified to family using NAWQA and EPA protocol for macroinvertebrate sorting and identification. We used Voshell (2002) *A Guide to Common Freshwater Invertebrates of North America,* Merritt et al. (2008) *An Introduction to the Aquatic Insects of North America 4th Edition, and* Peckarsky et al. (1990) *Freshwater Macroinvertebrates of Northeastern North America* to aid in the identification of benthic macroinvertebrate families.

<u>Community data analysis</u>

We assessed community composition using two approaches, *a)* the traditional biomonitoring rapid assessment approach focusing on composition at the order level (e.g. percent EPT) and *b)* relative abundance of one tolerant and intolerant three families based on NHDES and Carlisle et al. (2008) tolerance values. We graphed site-level family richness and relative abundance for each site over the three sampling periods. We then evaluated the relationship between order and family-level metrics and a suite of potential explanatory variables using multiple linear regression. The lack of a clear and consistent relationship between chloride and the community metrics in 2016 prompted us to take a multiple linear regression approach to better understand the influencers of the observed macroinvertebrate communities (Mazzone

2018). We took a stepwise parameter selection approach that included the following explanatory variables: discharge, stream area, as well as snapshot measures of water temperature (snapshot), pH, dissolved oxygen, chloride, and sodium. Sample month was also included because there is uncertainty in the timing of emergence for all families observed. We included data from the 2016 season to provide a multi-year assessment. The metrics and explanatory variables found in the best fit models of each are summarized in Table 1. In addition, we graphed a comparison of cumulative measures of family level richness (within and across orders) and relative abundance of indicator families observed in rock baskets retrieved during September/October sampling and kicknet samples from the same sampling period.

Table 1:

		Explanatory Variables										
	Response Variables	**Site**	**Month**	**Year**	**Chloride**	**DO**	**pH**	**Reach Area**	**Latitude**	**Water Temp**	**Elevation**	**Fluoride**
Common Biomonitoring Metrics	**Total Family Richness**	x	-	+	+	-	+					
	EPT Taxa Richness		-	+				+	+	+	+	
	Ephemeroptera Family Richness	x	-							-		
	Plecoptera Family Richness		-	+					+			
	Tricoptera Family Richness					-	+					+
	% EPT			+				+				+
	% Ephemeroptera		-					+		-	+	-
	% Plecoptera	x	-					-				
	% Tricoptera		+	-	+			-	+	+		
	% Chironomidae								-			
	% Diptera	x			-					-		
Family Level Responses	**Chloroperlidae Relative Abundance**[IT]	x	+									
	Leuctridae Relative Abundance[IT]		-		+							
	Philopotamidae Relative Abundance[IT]	x										+
	Simuliidae Relative Abundance[T]	x	-	+								
	Rhyacophiidae Relative Abundance[IT]	x										
	# Positive		2	5	3	0	2	3	3	2	2	3
	# Negative		8	1	1	2	0	2	1	3	0	1
	Total	**8**	**10**	**6**	**4**	**2**	**2**	**5**	**4**	**5**	**2**	**4**

<u>HSP70 expression</u>*:*

To test the utility of HSP70 in *Acroneuria* (Plecoptera, Perlidae) nymphs as a sub-lethal indicator of salt stress, our research sought to determine whether different combinations of NaCl dosage, temperature and time would cause differences in HSP70 expression. To answer this question, we conducted exposure experiments, exposing Perlidae nymphs to various levels of

NaCl and higher temperatures, then quantified the HSP70 response using Western blotting. Western blotting allows for the quantification of gene expression by measuring protein abundance from a given tissue. We predicted that HSP70 expression would show a marked increase with higher dosages of NaCl, independent of trial temperature, with higher levels attained over longer exposure times. Additionally, we predicted that interactions of higher temperature and NaCl dosages would result in the greatest expression of HSP70. The result of this approach was a measure of HSP70 related to experimental treatments and exposure time that could be assessed statistically.

Our local stream site for Perlidae collection was Clay Brook, Plymouth, NH (see Figure 9). This site was chosen for the high abundance of perlid stoneflies observed in previous seasons. Being a short drive from the laboratory, it also provided short transport times for live nymphs, reducing the chance of HSP70 induction through handling. Sampling of stream ion concentrations showed a low level of Cl^- (4.03 and 4.83mg/L, July and September 2017), suggesting little present influence of NaCl.

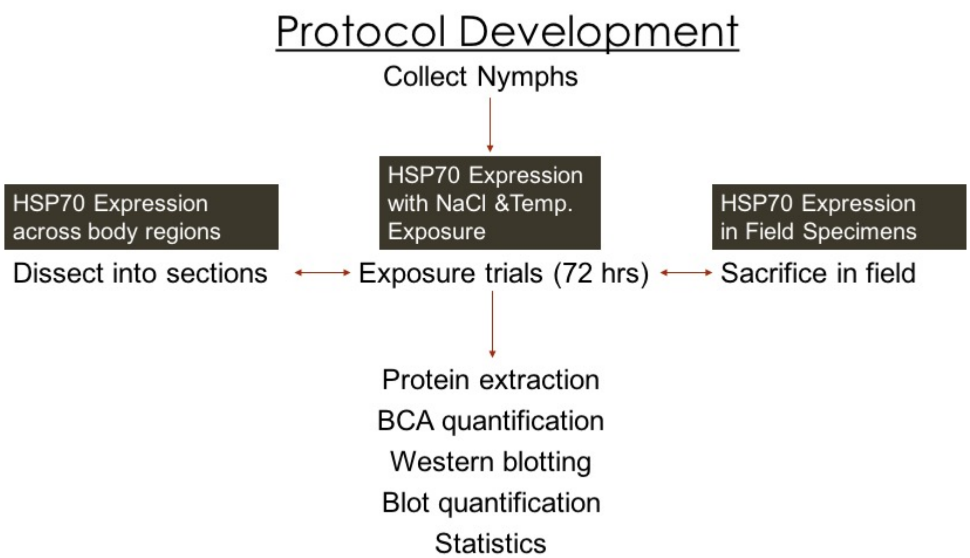


Figure 2: Overview of HSP70 analysis

We collected stonefly nymphs from May through July 2017 from Clay Brook using kicknetting, with stream temperature recorded during each visit. Nymphs were transported to the laboratory in a portable cooler to avoid heat stress. Stream water and leaves were collected from the site for micro aquaria to maintain consistent pre-treatment ionic conditions and provide a food source. Specimens and water were transferred to micro aquaria setups consisting of one-liter beakers with battery powered-bubblers as tanks for collected stoneflies (Kennedy et al. 2004; Echols et al. 2013). Screen netting was fitted into each tank to provide an attachment substrate. We exposed stonefly nymphs to various levels of NaCl and temperature (see Table 2). We acclimated nymphs for 72 hours at 4 and 21ºC prior to trials to rule out HSP70 expression due to handling/travel. Total protein was extracted from five nymphs to provide a measure of baseline HSP70 expression. Nymphs were exposed in a series of trials to 0 mg/L, 2500 mg/L, and 4000 mg/L NaCl dissolved in 50mL of diH_2O. Specimens were also exposed to temperatures of 4, 21, and 28°C. These temperatures and NaCl dosages were chosen to elicit a stress response without lethal harm to the experimental population. Following each application, nymphs were sacrificed at the 1, 24, 48, and 72 hour marks. Head samples were used for all blotting and analysis in this experiment following observed HSP70 concentration within this body region. Refer to Chapter 2 in Fruit (2018) for a detailed discussion of this preliminary work.

Figure 3. Location of Clay Brook, sample site for Perlidae in Plymouth, NH

Quantification of HSP70

Western blotting is a research technique for identifying target proteins from a mixture. Electrophoresis through a gel medium is used to separate extracts based on molecular weight/size. Proteins are then transferred to a membrane, resulting in bands of protein which can be identified by incubation with a primary and secondary antibody and subsequent substrate development (Mahmood & Yang, 2012). Blots were visualized using a BioRad ChemiDoc™ XRS+ imaging system (Bio-Rad Laboratories, Inc.), at which point images were exported as high resolution images. For more specific protocol, please refer to Fruit (2018).

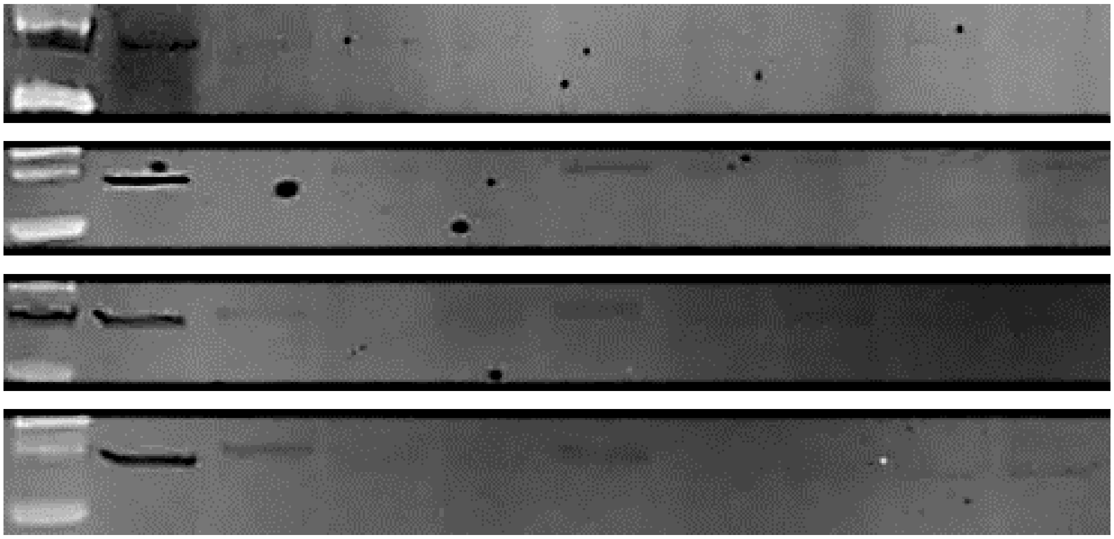


Figure 4. Representative western blots of head samples extracted 1, 24, 48 and 72 hours after treatment conditions commenced (ascending order from top).

We used ImageJ software to quantify pixel counts of HSP70 bands. Our method was similar to that of Taylor & Posch (2014), using a control sample loaded onto every blot to standardize between all samples. Background noise from the target protein of each sample was subtracted in ImageJ, and the resulting output was multiplied by a ratio of the loading control for each sample and the inter-blot control. This normalized value is referred to as the normalized density to the loading control (NDL). The NDL of each experimental sample was then divided by the NDL of each inter-blot control, yielding a fold difference (FD) value from the amount of control expression (Taylor et al. 2013; Taylor & Posch, 2014). We used a sample of *Drosophila* protein in this study as the inter-blot control and our positive control. For the loading control of each experimental and the inter-blot sample, we used total protein of each lane stained by Ponceau-S. Hereafter, FD values of experimental samples will be referred to as HSP70 expression.

Trial Time (hrs)	N	NaCl (mg/L)	Temperature (℃)
0	5	0	4
1	2	2500	4
	4	4000	4
	1	0	21
	2	2500	21
	2	4000	21
	3	0	28
	2	2500	28
24	2	4000	4
	2	0	4
	1	2500	21
	2	4000	21
	2	0	21
	1	2500	28
	2	4000	28
48	1	0	4
	2	2500	4
	2	4000	21
	2	0	21
	2	2500	21
	2	4000	28
	1	0	28
72	2	2500	4
	3	4000	4
	2	0	21
	2	2500	21
	2	4000	21
	3	0	28
	2	2500	28
TOTAL	61		

Table 2 - Total Salt Trial Subjects. Including trial time, temperature and NaCl dosage and number of individuals per treatment.

<u>Data analysis</u>

We had 61 samples in total, for multiple combinations of 5 different trial times, 3 different NaCl dosages and 3 different trial temperatures. Observed HSP70 expression ranged from FD values of -0.0592 (compared to observed control) to 0.589, nearly a ten-fold difference across treatments (see Appendix C). We graphed box-plots to illustrate differences in HSP70 across trials representing unique combinations of NaCl dose, temperature and exposure time. To best visualize all treatment groups, trial temperature and NaCl treatments were concatenated and used as one factor alongside trial time (see Figure 11). We created ANOVA interaction plots visualizing model effects and mean distributions of HSP70 expression indicating interactions of combinations of explanatory variables. We then graphed boxplots of HSP70 expression in relation to time and either NaCl or temperature to visualize which factors, if any, corresponded (Figure 5). To verify observed effects, we ran non-parametric ANOVA (Kruskall-Wallis) tests to test for statistical differences in observed HSP70 expression explained by NaCl dose, temperature and exposure time in isolation. Each experimental variable was run independently, with other variables collapsed. To address the possibility of factors interacting to influence variation of HSP70 expression, two-way ANOVA was used to examine variation with respect to combinations of NaCl dosage, trial temperature and exposure time. Finally, multiple-linear regression was used to corroborate ANOVA results for the observed relationship of these factors on variation in HSP70 expression.

Principal Findings & Significance

Chloride concentrations

Monthly snapshot water chemistry samples confirmed the initial classification of streams (based on snapshot sampling in 2013 and 2014) and were similar to those reported from 2016, with the exception of consistently lower concentrations at the Beaver Brook site (BBU) in Keene, NH. The new site for 2017 turned out to be another low concentration site, despite extremely close proximity to road. As also seen in 2016, Wednesday Hill Brook (WHB) had the highest Cl concentrations across our study sites and over time. Even this, our highest observed chloride concentration, was substantially below the EPA's chronic toxicity concentration of 230 mg/L. Chloride concentrations increased at most sites between July and September/October, which we believe is attributed to lower water levels of which groundwater likely comprises a larger portion of stream water. These findings support the findings of Daily et al. (2009).

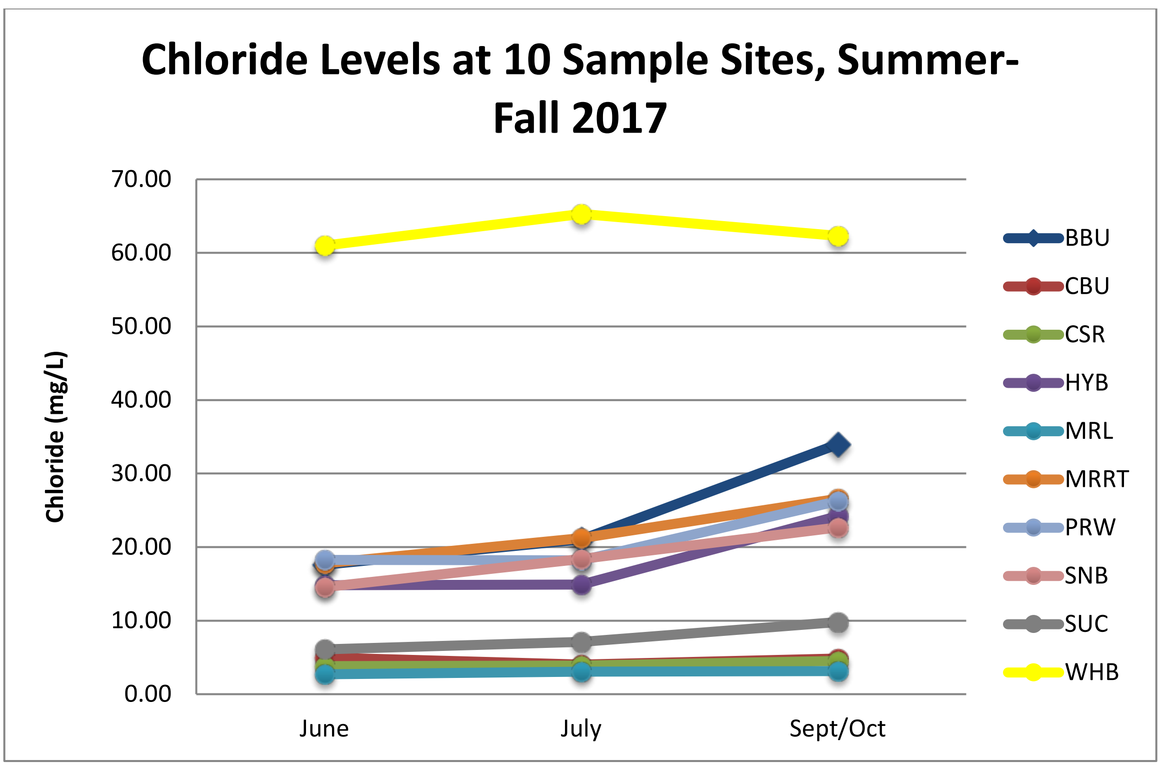


Figure 5: Snapshot sampling of chloride in 10 sample streams between June and September/October 2017.

Community Composition

We found that chloride rarely explained a significant portion of the observed variation in the aforementioned community metrics in summers 2016 & 2017 (Table 1). Of the four metrics that were significantly related to chloride, only % Diptera was negatively related to chloride, which was opposite the expectation. Among the unexpected, yet significant relationships with chloride were positive observed relationships with the percent Plecoptera (Figure 6). Further analysis indicated that this pattern, also observed in 2017, was largely driven by the relative abundance of Leuctridae, a plecopteran family previously categorized as intolerant to poor water quality (Figure 7). Interestingly, few other Plecoptera families were present when chloride concentrations exceeded 30 mg/L and this was evident with the negative trend observed between chloride and Plecoptera richness (Figure 8). We had adopted Leutridae as an intolerant indicator family based on published tolerance scores. Our observations don't support the perceived sensitivity of this family. This may be driven by unknowingly sampling a single genus or species that is more tolerant than others in the family. Alternatively, it could suggest that Leutrids are less sensitive to ionic concentrations than other sources of water quality stress, which would imply that they are a poor bioindicator choice for monitoring the effects of salinization.

We observed was significant inter-annual and intra-annual variability with generally higher metric values in 2017 and lower values as the season progressed from June to Sept/October. Ephemeroptera richness, percent Ephemeroptera, and percent Diptera were negatively related to observed water temperatures, whereas percent Tricoptera and EPT Richness were positively related to observed water temperatures. This suggests that Ephemeroptera composition may serve as a strong bioindicator of water temperature stress, even at the relatively low temperatures observed during our study. We did not find significant relationships between chloride or temperature and the majority of our pre-identified indicator families, with the exception of Leuctridae.

Overall, our observed communities seem to be shaped more by natural site-level variability in elevation, latitude, reach area, and time than by ionic concentrations or temperature, suggesting that chloride concentrations are not negatively affecting the streams in this study.

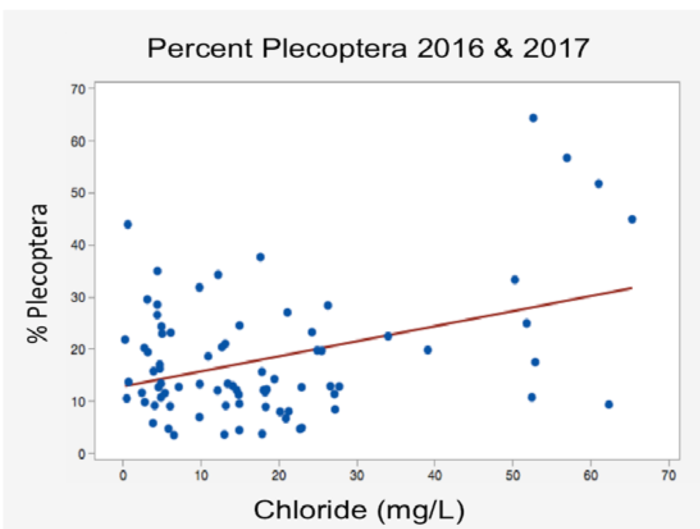


Figure 6: Relative abundance (%) of Plecoptera individuals

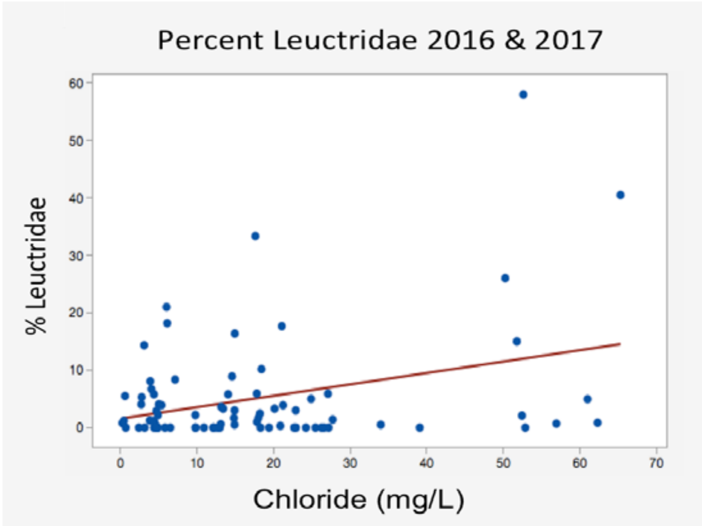


Figure 7: Relative abundance (%) of Leuctridae individuals (order: Plecoptera)

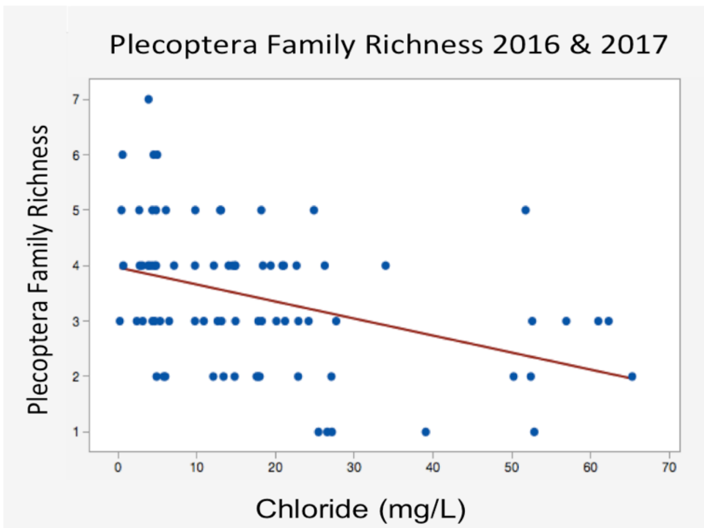


Figure 8: Plecoptera richness (i.e.number of Plecoptera families)

Rock baskets vs. Kicknet sampling

We compared communities observed using kick netting and rock basket methods to assess whether important metrics were equally represented. Our results suggest that kick netting with 10 sets over 100m yielded higher total abundance of macroinvertebrates and higher richness of all families, EPT families collectively and by order at most sites (Figures 9-14). The difference in richness measures is clear evidence that the rock baskets do not reflect the full diversity within a defined reach. While this is not completely necessary, depending on the objective of the assessment, it could be problematic if the method is biased away from potential indicators of chloride stress. For example, individuals in the Philopotamidae and Simuliidae families were rarely found in a rock basket sample (Figures 13 & 14). The lack of taxanomic representation in rock baskets will also influence the relative abundance of those groups present. For example, percent Chironomidae (was consistently much higher in rock basket than kick net samples at our sites (Figure 15). We also noticed that difference between the rock basket and kick net samples was among the greatest at Wednesday Hill Brook (WHB), the site with consistently higher chloride concentrations. In pursuit of a reliable bioindicator taxa, we must also consider the best method for sampling.

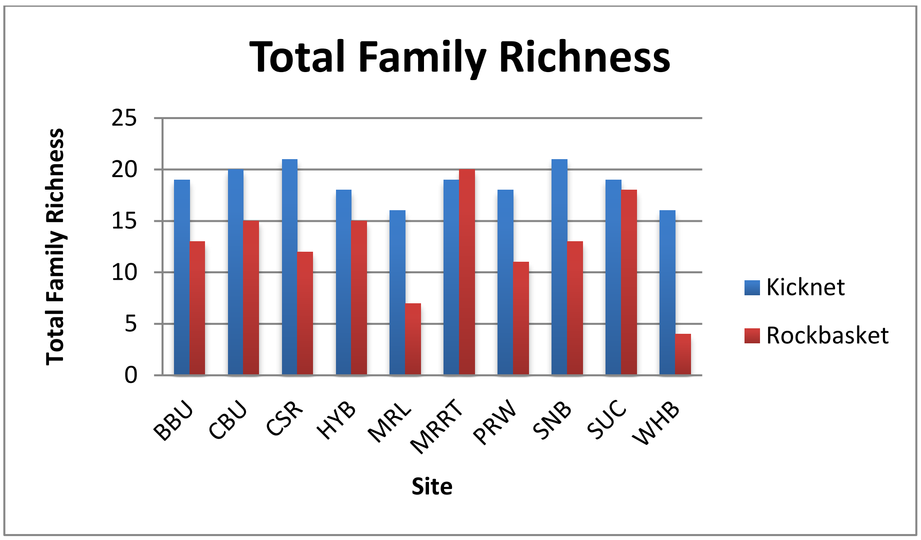


Figure 9: Total family richness measured using kicknet and rock basket sampling methods.

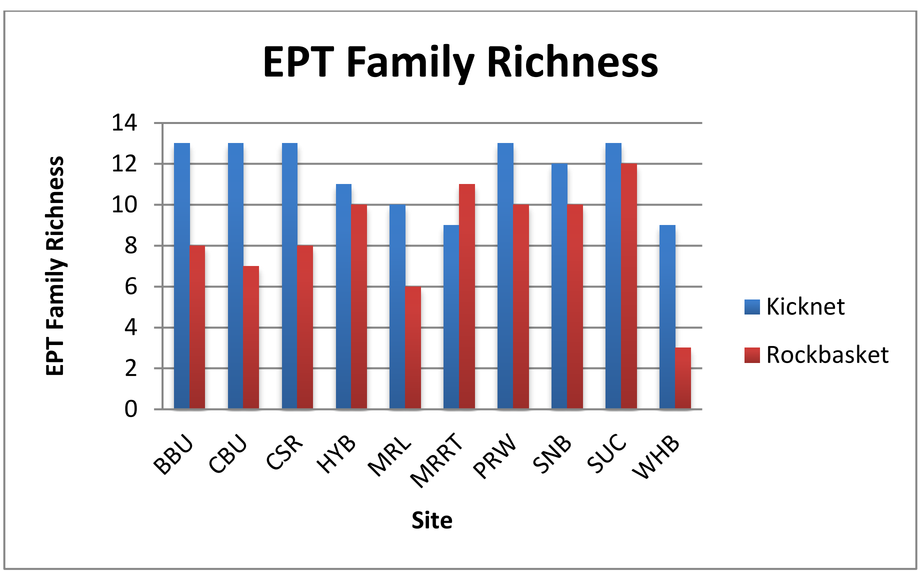


Figure 10: EPT family richness measured using kick net and rock basket sampling methods.

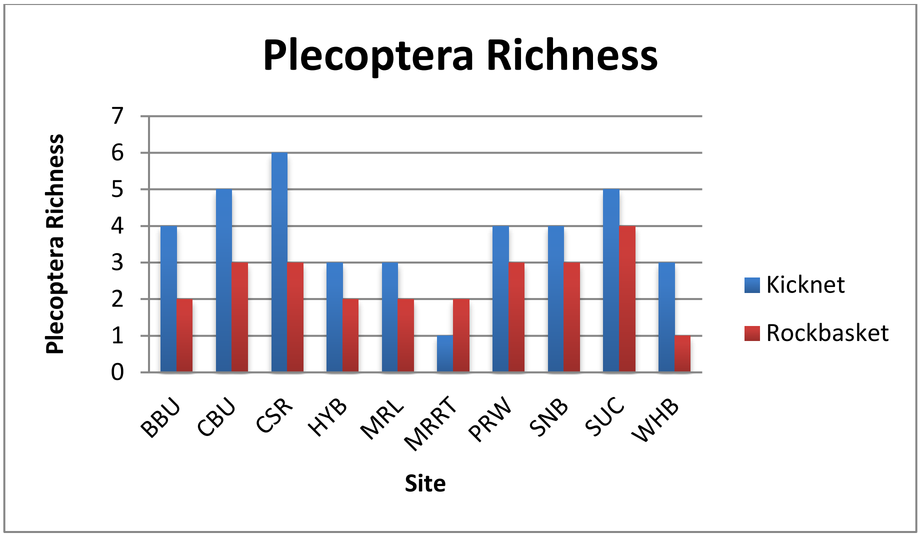


Figure 11: Plecoptera richness measured using kick net and rock basket sampling methods.

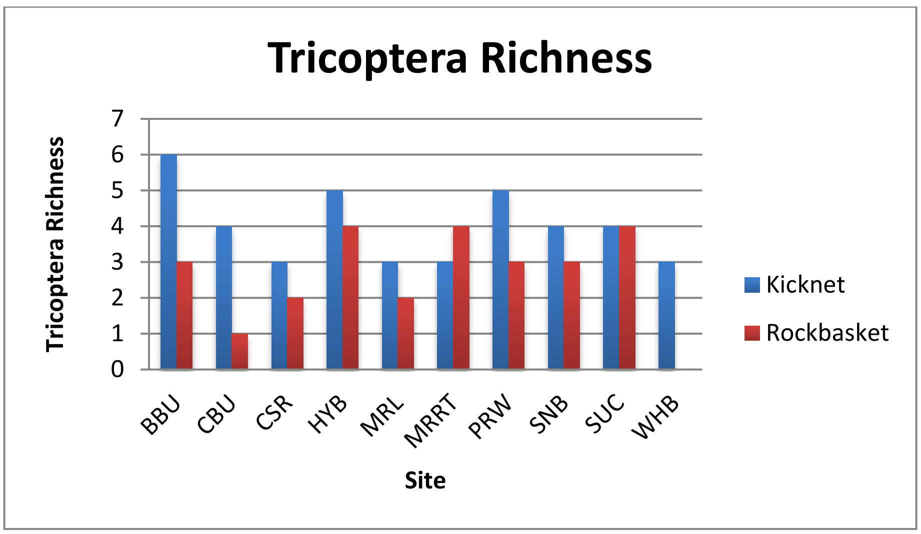


Figure 12: Tricoptera richness measured using kick net and rock basket sampling methods.

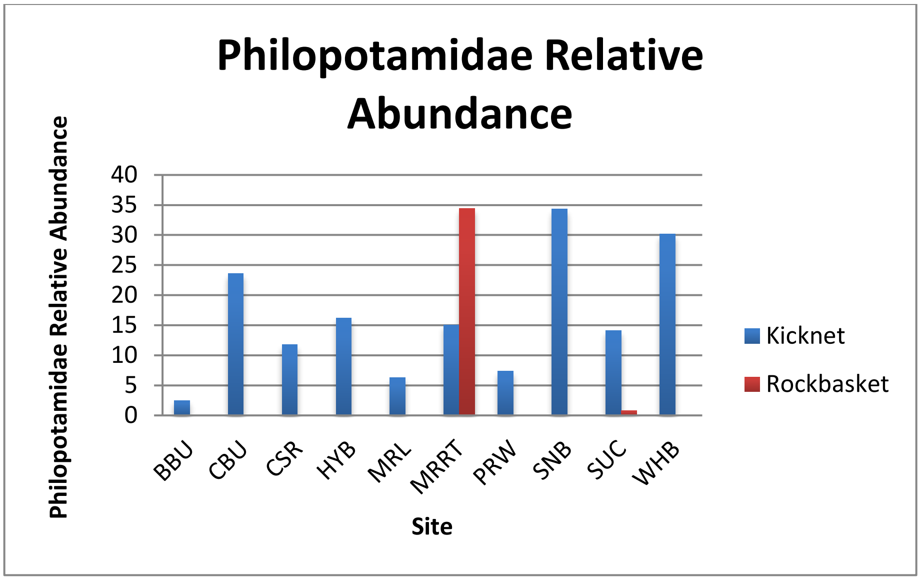


Figure 13: Relative abundance of Philopotamidae measured using kick net and rock basket sampling methods.

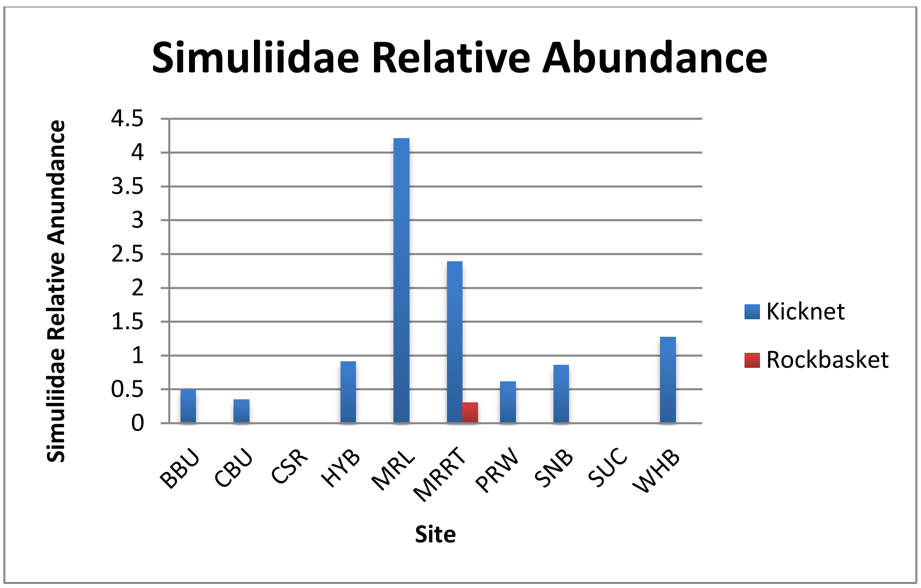


Figure 14: Relative abundance of Simuliidae measured using kick net and rock basket sampling methods.

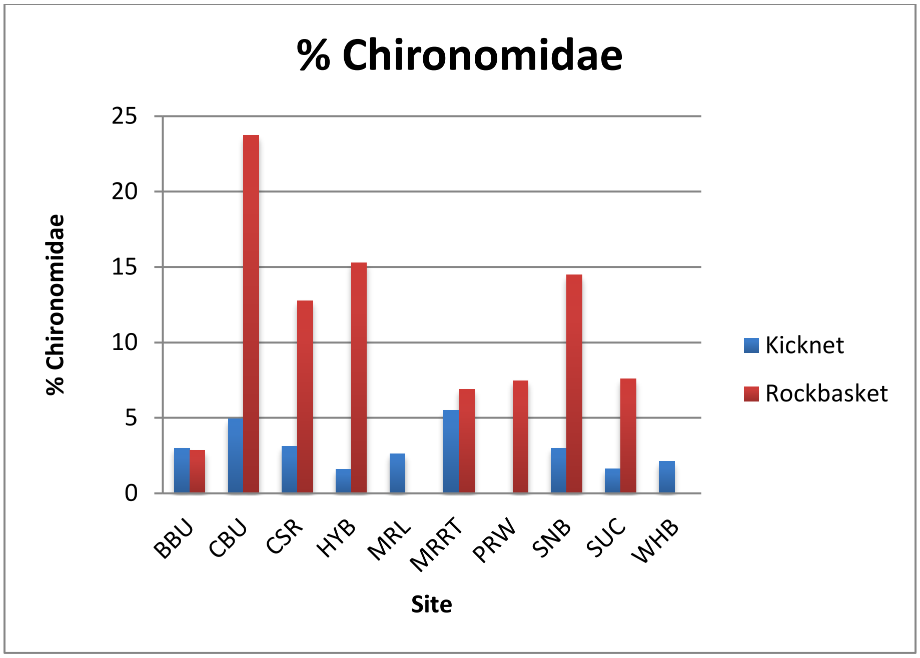


Figure 15: Relative abundance (%) of Chironomidae measured using kick net and rock basket sampling methods.

<u>HSP Analysis</u>

Alone, NaCl and temperature variables did not explain the observed variation in HSP70 expression (N = 61, p-value = 0.9548 and N = 61, p-value = 0.3508); however, there was an observed and disproportionate uptick in HSP expression at for samples held at 21 and 28° C (Figure 16).

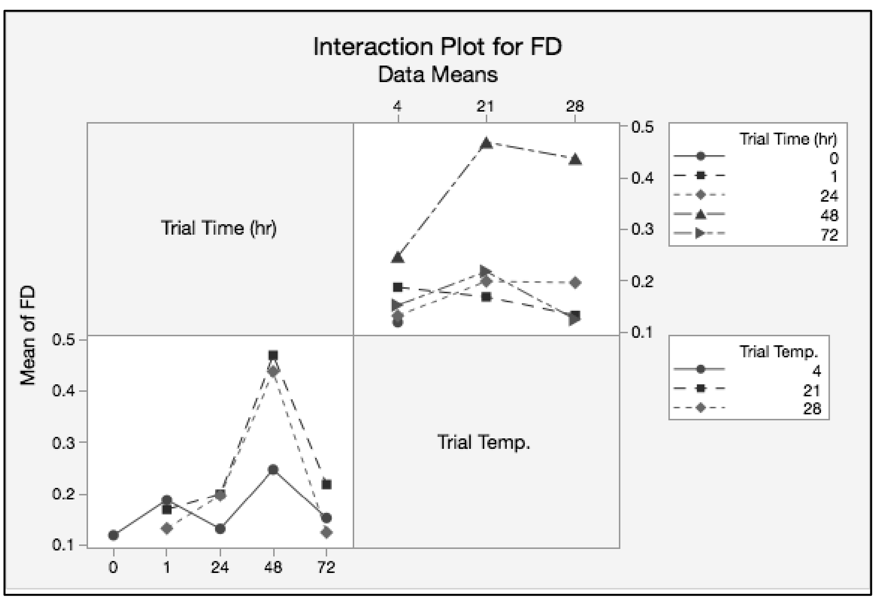
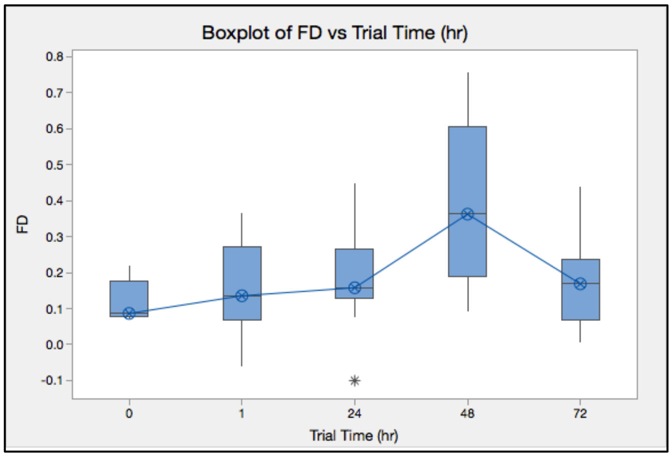


Figure 16: Interaction plot illustrating the observed relationships between exposure time and temperature.

Exposure time was a significant treatment factor (N = 61, p-value = 0.0194). This was supported by observed levels of HSP70 expression after 48 hours (Figure 16 and 17). Likewise, the two-way ANOVA analysis also suggested exposure time partially explained the increase in HSP70 expression (N = 59, p-value ≅ 0.0044). The results from our various ANOVA analysis were corroborated by running a multiple linear regression of NaCl dosage, trial temperature and exposure time as a categorical variable in which 48 hours of exposure time was found to have significantly higher HSP70 expression (N = 60, p-value ≅ 0.005).



Test

Null hypothesis H_0: All medians are equal
Alternative hypothesis H_1: At least one median is different

DF	H-Value	P-Value
4	11.73	0.0194

Descriptive Statistics

Trial Time (hr)	N	Median	Mean Rank	Z-Value
0	5	0.086489	20.8	-1.34
1	16	0.135557	27.0	-1.05
24	12	0.157863	28.8	-0.47
48	12	0.362314	46.2	3.30
72	16	0.168677	28.4	-0.67
Overall	61		31.0	

Figure 17 - Kruskal-Wallis ANOVA results showing significant differences between 48hr extractions and other time periods.

Neither NaCl or temperature explained the variation observed in HSP70 expression at the treatment levels tested; however, our results do indicate that exposure time explained a significant portion of observed variability in the HSP70 expression in stonefly nymphs. Expression noticeably increased in specimens exposed to 21 or 28°C, peaking between 24 to 48 hours or 48-72 hours and returning to baseline levels by 72 hours (backed by Kruskal-Wallis ANOVA, Two-Way ANOVA and Multiple-Linear Regression).

Response to NaCl

The lack of a statistically significant treatment effect could be explained by several factors, one of which is the particular chemical composition to which specimens were exposed. Molecular grade NaCl was used as a proxy for road salt; however, this lacks many of the additives used for road deicing (such as abrasives) which may have synergistic effects harmful to aquatic macroinvertebrates. Additionally, NaCl (or free Cl- ions, for that matter) may not be as acutely toxic to aquatic macroinvertebrates as previously thought. Instead, the toxicity may be attributed to other free ions released from soil sediments by the constituents of road salts. NaCl has been shown to mobilize metals within soils, both through complexing with Cl^- ions and cation exchange with Na^+ ions (Benjamin, 2002; Norrstrom and Jacks, 1998; Backstrom et al. 2004). A third potential explanation might be that aeration from the bubbler in the micro aquaria reduced exposure to toxicants as compared to stagnant/stiller water (Sanders & Cope, 1968.) This was unavoidable in our experiment, as we sought to recreate stream conditions as closely as possible. A final potential explanation was the small sample size of each specific combination of trial factors preventing a proper analysis of NaCl-related stress. This was due to the various temperatures and exposure times, meaning only one to four specimens per NaCl dosage; ideally, each NaCl dosage would have at least 20 representative specimens.

Response to temperature

The temperature component of these trials is also interesting and unexpected. Our results indicate that specimens exposed to 21°C reached the highest levels of HSP70 expression at 48 hours, while those exposed to temperatures of 28°C achieved lower levels of HSP70 expression at 48 hours and fell to lower levels than both 4 & 21°C specimens after 72 hours. This was based entirely off data visualization and means observed in interaction plots, as statistical tests discounted temperature as significantly influencing variation in HSP70. At times, organisms exposed to the high levels of harmful contaminants in an experiment have lower levels of HSP70 expression than those exposed to lower levels (Pyza et al. 1997 & Kohler et al. 1992). Similarly, Pyza et al. (1997) found the greatest mean HSP70 levels in heat-treated centipedes to be at 15°C, and not at 5 or 25°C. The HSP70 response to temperature is well-known, but still subject to variability. Threshold temperatures for the activation of HSP genes is known to vary over the lifetime of an individual and is subject to thermal acclimation to an environment (Buckley et al. 2001). Brook Trout, for example, express high levels of HSP70 at the same average temperature across two different years of study (Chadwick Jr. et al. 2015). This could suggest that past exposure to temperature stress had hardened nymphs against physiological stress from heat. More specifically, it may be that expression of HSP70 in stonefly nymphs under these experimental conditions was exhausted by temperatures of 28°C after an attempted spike to achieve homeostasis by 48 hours, and subsequently crashed by 72 hours. The observation that those specimens' HSP70 expression exposed to 21°C rose higher at 48 hours and finished higher at 72 hours could suggest that this temperature exposure was not severe enough to exhaust the HSP70 response.

Alternative Influences

The importance of the exposure history of individuals should not be overlooked in a study using HSPs. While we attempted to minimize any stressors influencing the expression of HSP70, it is difficult to completely account for past influences. Hochachka & Somero (1984) point out that there is significant adaptive variation of the heat shock response from recent thermal history and selective forces. Moreover, the threshold of HSP induction varies due to thermal acclimation, and can vary over the lifetime of a single individual. HSP70 can build up within cells following repeated gradual warming events (Buckley et al. 2001).

A related problem lies with the possibility of individual variation of the heat shock response among organisms collected in the same environment. This is because past exposures to stressful conditions are capable of acting as evolutionary forces upon populations (Sørensen et al. 2003). Feder & Hofmann (1999) point out that while variation in HSPs can be due to seasonal variation or acclimation to stressors, natural variation will also be present from genetic differences of individuals. In addition, variation in the tolerance to ionic changes may manifest at the species level, rather than the genus level at which we worked.

Conclusion

Evidence from our two-year study suggests that streams in central New Hampshire remain relatively unstressed by salt additions attributed to road salt. All streams monitored were under the EPA's chronic toxicity concentration. However, our study has found that not all

Plecoptera taxa are “sensitive” or intolerant to salinity stress. We found a positive relationship between Leuctridae (family in order of Plecoptera) and chloride concentrations during both study seasons, despite a decrease in Plecoptera family richness. Further, the molecular level analyses found that *Acroneuria* (another genus in the family of Perlidae in order of Plecoptera) had an exceptionally high tolerance to NaCl in lab settings (4000 mg/L). This leads us to believe that sensitivity to salt stress likely varies at the family, if not genus/species, level. If so, traditional biomonitoring metrics that focus on the relative abundance of EPT taxa may not be fine enough resolution to detect stress. Finally, our comparison of benthic macroinvertebrates detected in rock baskets to that of kicknets suggest that rock baskets do not fully reflect the biota present and that they may select against families/genera with salt sensitivity by nature of the method alone. Further research is needed to compare rock basket taxa to kicknet and to understand which families are more vulnerable to salt stress in New Hampshire. To accomplish such, we suggest more studies in areas where chloride concentrations are higher – mainly in southern NH; however, it is also important to continue to monitor streams in central and northern NH to maintain healthy systems.

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Notable Awards:

Dr. Amy Villamagna was honored with the Helen Abbott Endowed Professors of Environmental Studies (2016-2020) for her research on the environment and engagement of students in research.

Katerina Crowley was awarded the Marapesse Scholarship in 2017 for her participation in this research on Sucker Brook, a tributary to Webster Lake (NH).

Katerina Crowley was awarded second place in student poster competition at the 2018 New England Association of Environmental Biologists in Devens, MA.

Publications and Presentations:

2018

Fruit, R. K. 2018. 'A Novel Approach to Biomonitoring: Identifying Communities At-risk from Salinization Using HSP70 Expression in Stonefly Nymphs'. Master of Science in Environmental Science & Policy. Plymouth State University. Plymouth, NH (USA)

Mazzone, M. 2018. 'The Impacts of Chloride on Macroinvertebrate Communities in New Hampshire Streams'. Master of Science in Environmental Science & Policy. Plymouth State University. Plymouth, NH (USA)

Fruit, R., A. Villamagna, B. O'Donnell. 2017. HSP70 Induction Following Salt and Temperature Stress in the Stonefly *Acroneuria* – A Novel Approach to Biomonitoring. New England Association of Environmental Biologists annual meeting (Devens, MA). Oral presentation

Kat Crowley. Assessing ecological stress from chloride in New Hampshire streams at community and population levels. New England Association of Environmental Biologists annual meeting (Devens, MA). Poster presentation

Fruit, R., A. Villamagna, B. O'Donnell. 2017. Identifying Communities at-risk from Salinization Using HSP70 Expression in Stonefly Nymphs. New Hampshire Water and Watersheds conference. Poster presentation

Kat Crowley. Assessing ecological stress from chloride in New Hampshire streams at community and population levels. New Hampshire Water and Watersheds conference. Poster presentation

2017 (2016-17 funding cycle)

Duquette, R., A. Villamagna, B. O'Donnell. 2017. *Assessment of Mayfly, Stonefly and Caddisfly abundances in relation to chloride in New Hampshire streams.* New England Association of Environmental Biologists annual meeting (Hartford, CT) *[poster printed but not presented due to poor blizzard travel conditions]*

Lafortune, T., A. Villamagna, B. O'Donnell. 2016. *Air and Stream Temperature Relationships and Influence on Macroinvertebrate Communities in New Hampshire.* New England Association of Environmental Biologists annual meeting (Hartford, CT) *[poster printed but not presented due to poor blizzard travel conditions]*

Fruit, R. , A. Villamagna, B. O'Donnell. 2017. *Quantification of HSP70 Expression in Mayflies: A Novel Bioindicator of Road Salt Pollution.* New England Association of Environmental Biologists annual meeting (Hartford, CT) oral presentation

Mazzone, M. A. Villamagna, B. O'Donnell. 2017. *Assessing Salt Stress In Selected NH Streams at the Community Level For Macroinvertebrates.* New England Association of Environmental Biologists annual meeting (Hartford, CT) *[oral presentation prepared but not presented due to poor blizzard travel conditions]*

Duquette, R., A. Villamagna, B. O'Donnell. 2017. *Assessment of Mayfly, Stonefly and Caddisfly abundances in relation to chloride in New Hampshire streams.* Plymouth State University Showcase of Excellence (poster)

Lafortune, T., A. Villamagna, B. O'Donnell. 2016. *Air and Stream Temperature Relationships and Influence on Macroinvertebrate Communities in New Hampshire.* Plymouth State University Showcase of Excellence (poster)

Fruit, R., A. Villamagna, B. O'Donnell. 2017. *Quantification of HSP70 Expression in Mayflies: A Novel Bioindicator of Road Salt Pollution.* Plymouth State University Showcase of Excellence (oral presentation)

Mazzone, M., A. Villamagna, B. O'Donnell. 2017. Assessing Salt Stress on Macroinvertebrate Communities in NH Streams. Plymouth State University Showcase of Excellence (oral presentation)

2016 (2016-17 funding cycle)

Fruit, R. , A. Villamagna, B. O'Donnell. 2016. *Stress Protein Expression: An Early Warning Sign of Freshwater Community Degradation via Road Salt Runoff in New Hampshire* (poster), 2016 NH Water and Watersheds Conference in Plymouth, New Hampshire

Duquette, R., A. Villamagna, B. O'Donnell. 2016. *Assessment of Mayfly, Stonefly and Caddisfly abundances in relation to chloride in New Hampshire streams.* Hubbard Brook Research Experience for Undergraduates Symposium (Thorton, NH)

Lafortune, T., A. Villamagna, B. O'Donnell. 2016. *Air and Stream Temperature Relationships and Influence on Macroinvertebrate Communities in New Hampshire.* Hubbard Brook Research Experience for Undergraduates Symposium (Thorton, NH)

Duquette, R., A. Villamagna, B. O'Donnell. 2016. *Assessment of Mayfly, Stonefly and Caddisfly abundances in relation to chloride in New Hampshire streams.* EPSCoR Research for Undergraduates Symposium at University of New Hampshire (Durham, NH)

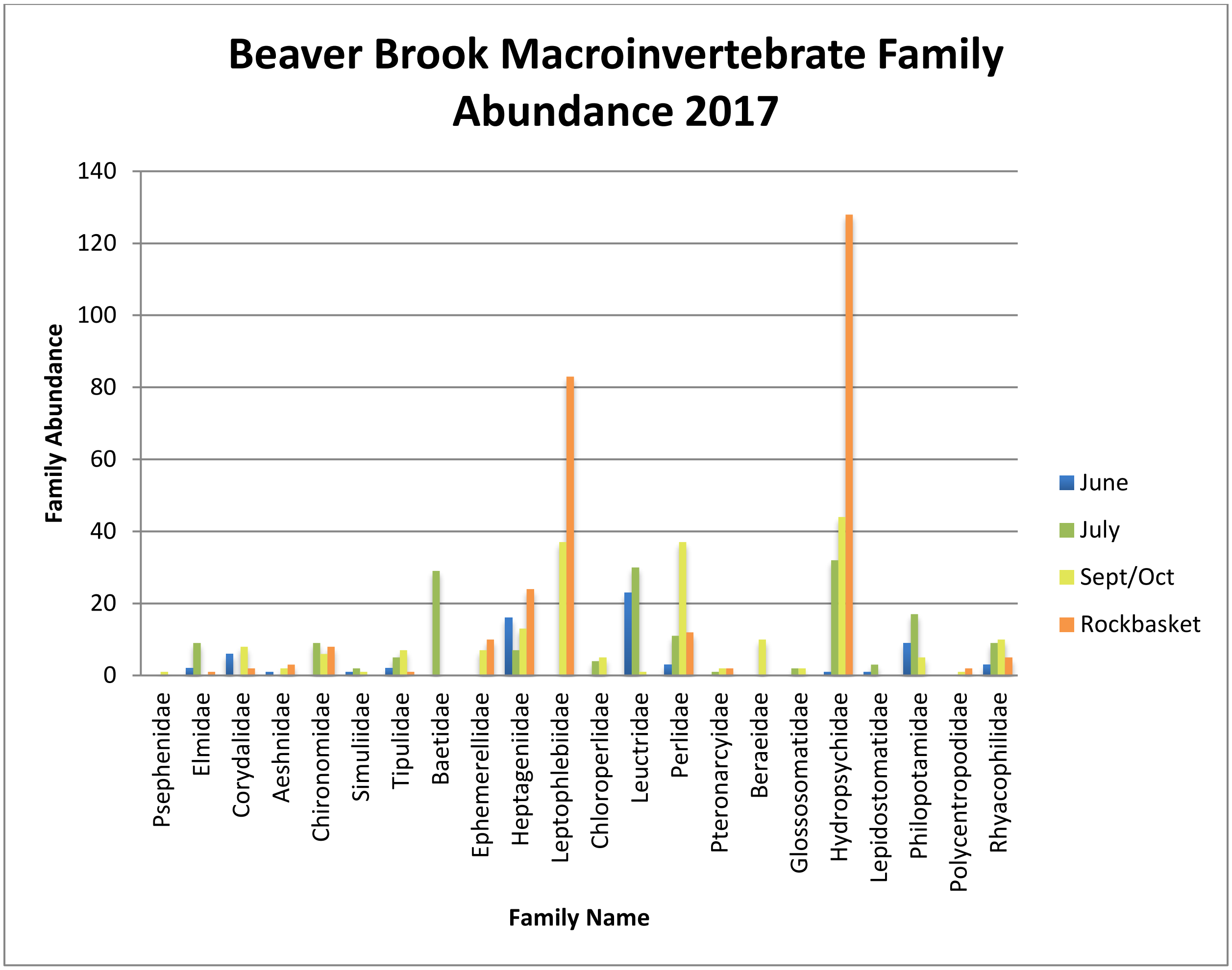
Lafortune, T., A. Villamagna, B. O'Donnell. 2016. *Air and Stream Temperature Relationships and Influence on Macroinvertebrate Communities in New Hampshire.* EPSCoR Research for Undergraduates Symposium at University of New Hampshire (Durham, NH)

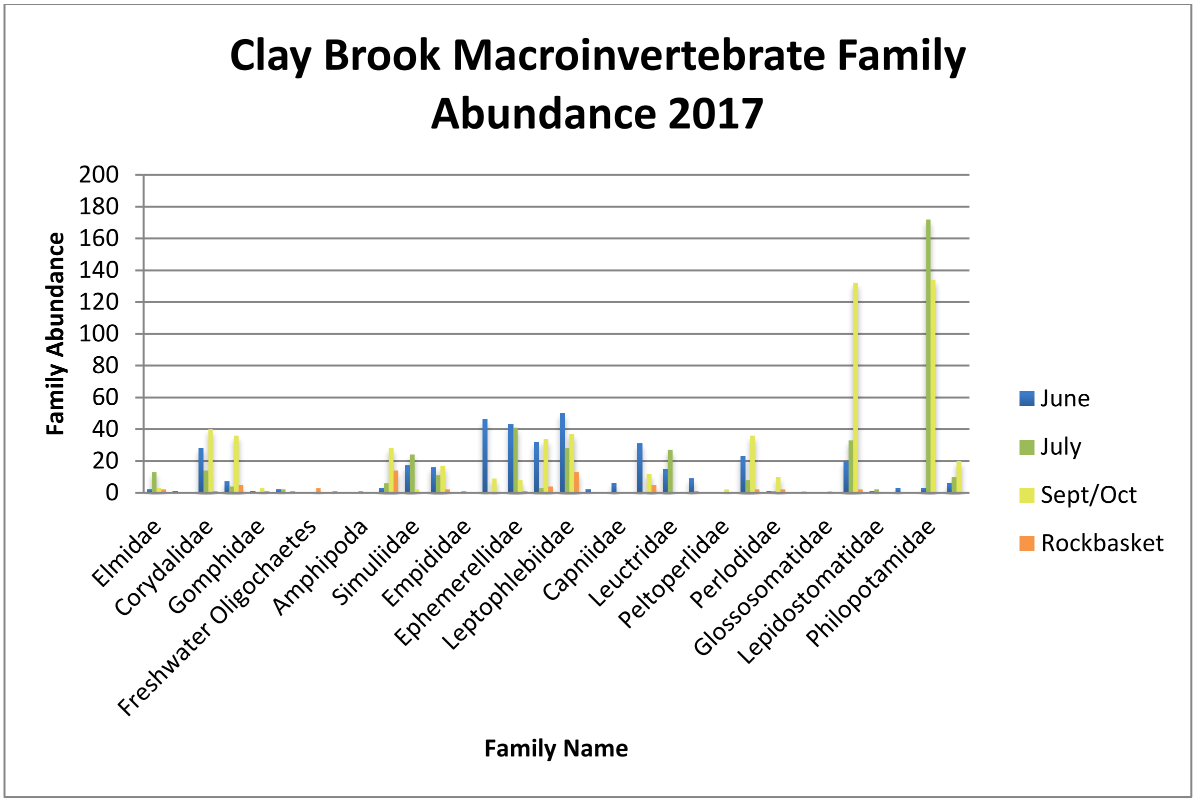
Number of students supported*:* 2 MS students, K. Crowley and M. Hirschler. 4 undergraduate students, S. Bevier, T. Lafortune, M. Conlon, J. Burdick were affiliated with the project through university match and research collaboration. Not all received direct funding from NH WRRC.

Number of faculty supported: Assistant professor, Amy Villamagna (Ph.D.) and Associate professor, Brigid O'Donnell (Ph.D.) were affiliated with the project through university match and research collaboration.

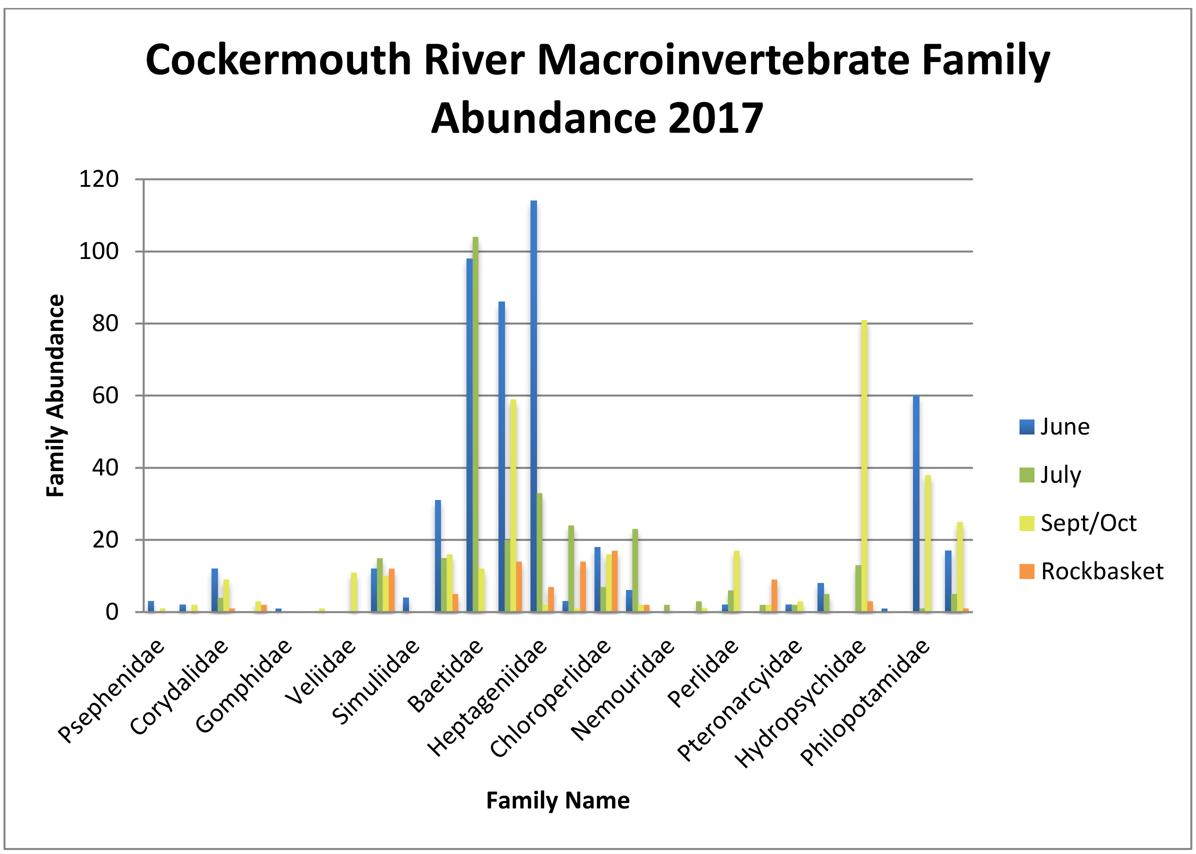
APPENDICES

Appendix A: Within season comparison of family level abundance observed through kicknet sampling for benthic macroinvertebrates at all ten study sites.

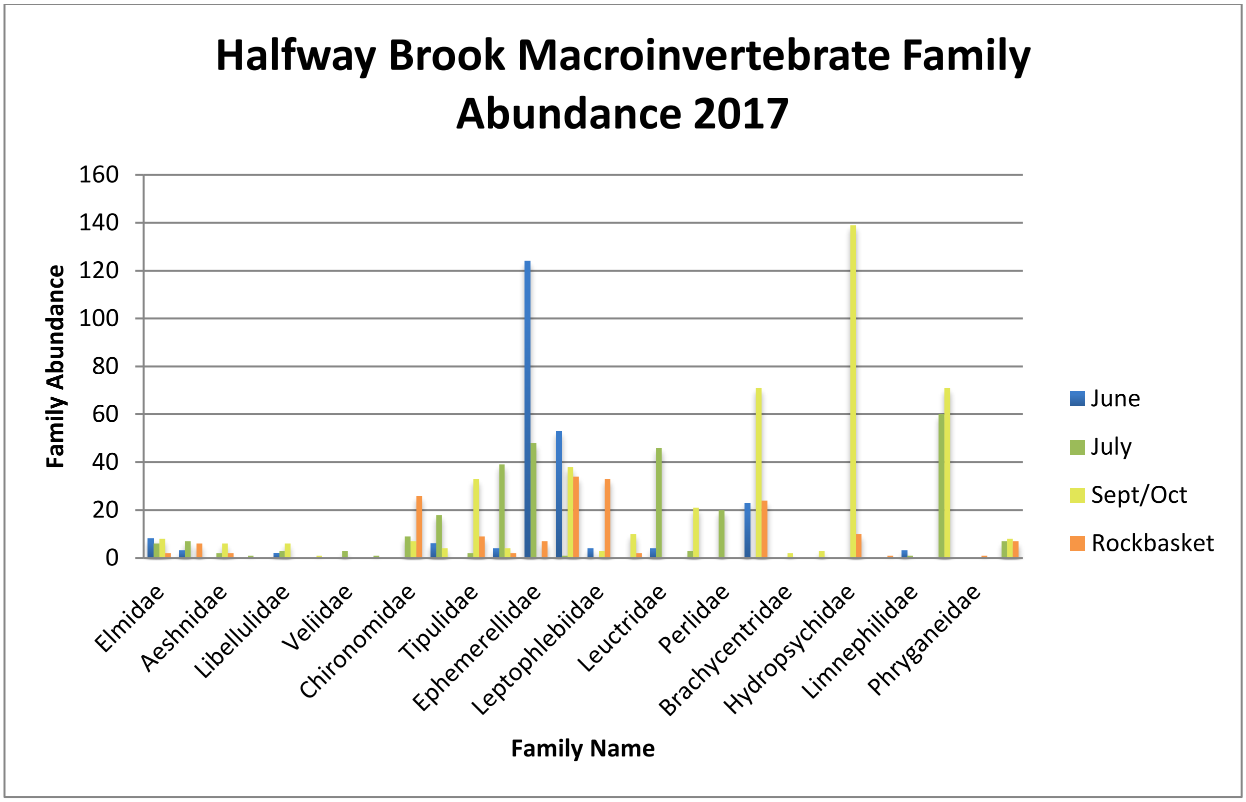


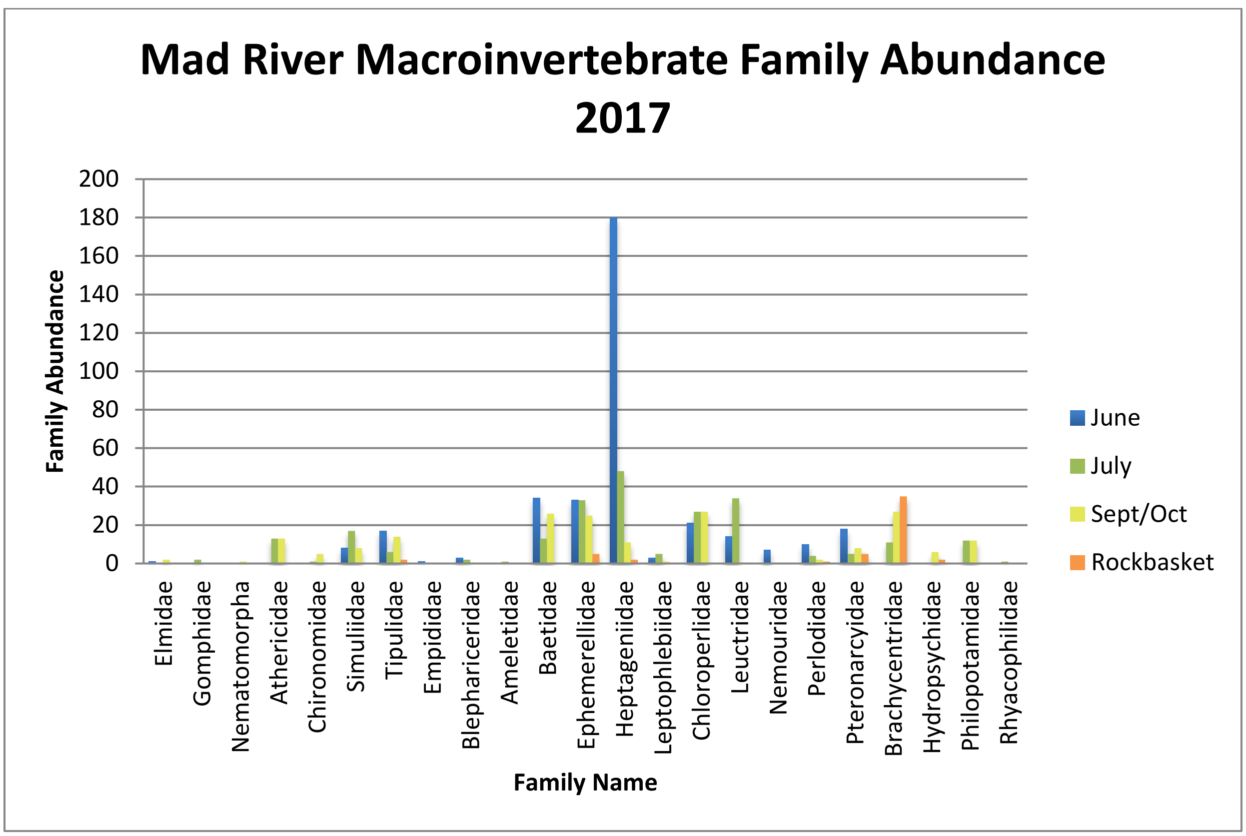


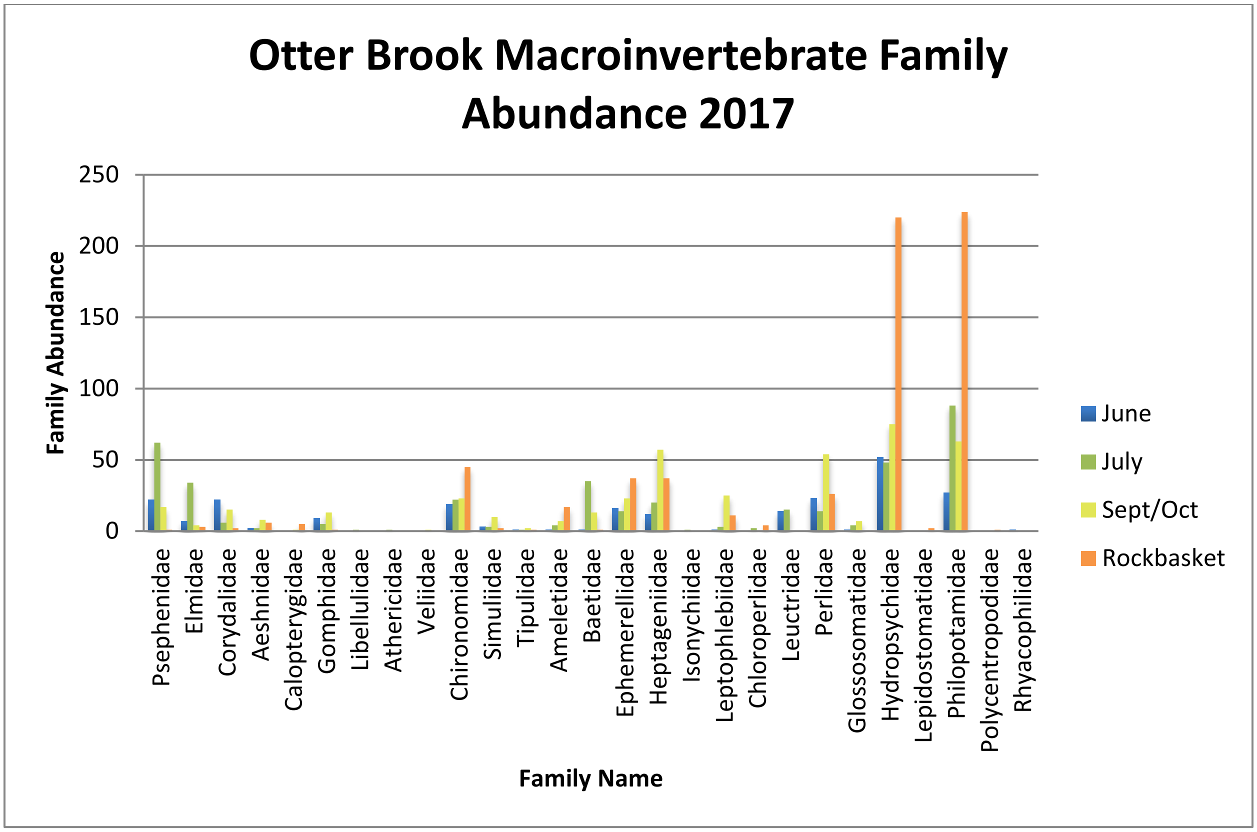
Clay Brook Macroinvertebrate Family Abundance 2017
200
180
160
140
120
100
80
60
40
20
0
Family Abundance
Elmidae
Corydalidae
Gomphidae
Freshwater Oligochaetes
Amphipoda
Simuliidae
Empididae
Ephemerellidae
Leptophlebiidae
Capniidae
Leuctridae
Peltoperlidae
Perlodidae
Glossosomatidae
Lepidostomatidae
Philopotamidae
Family Name
June
July
Sept/Oct
Rockbasket



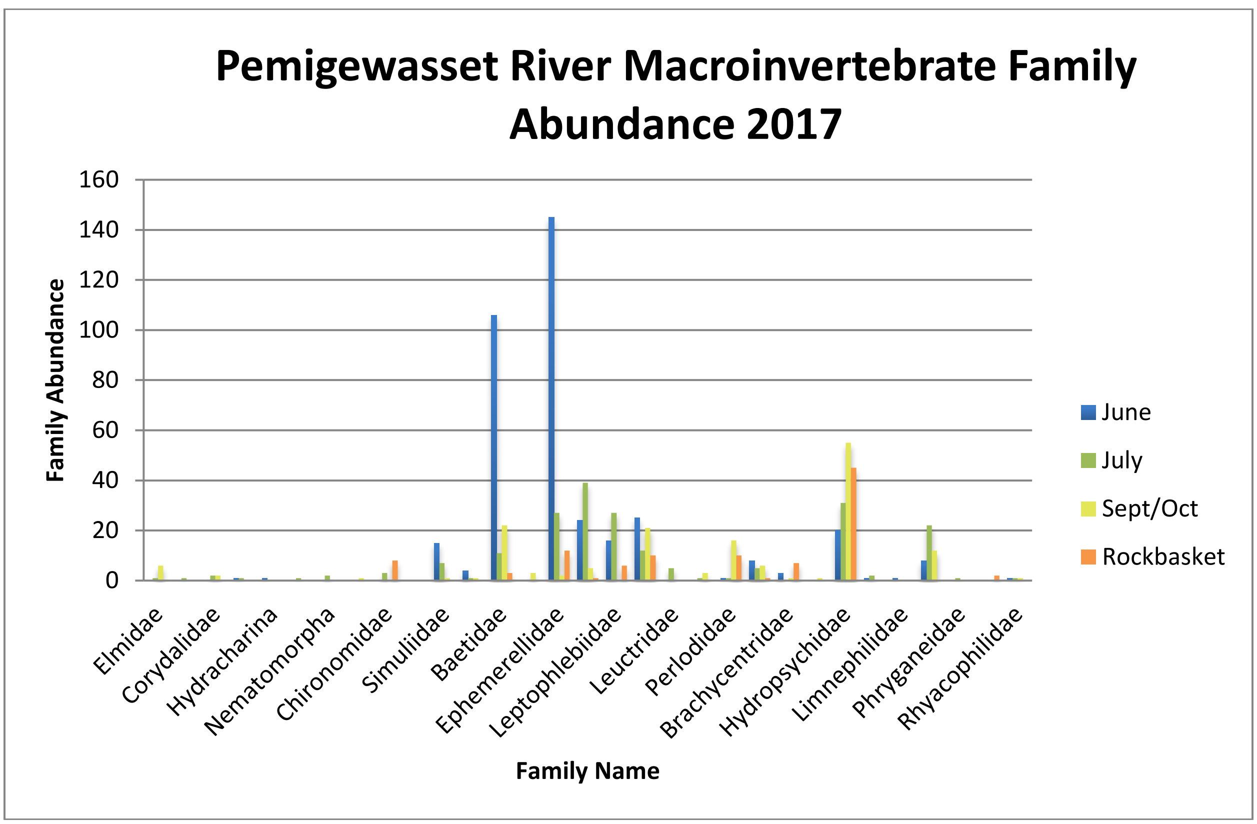
Cockermouth River Macroinvertebrate Family Abundance 2017
120
100
80
60
40
20
0
Family Abundance
Psephenidae
Corydalidae
Gomphidae
Veliidae
Simuliidae
Baetidae
Heptageniidae
Chloroperlidae
Nemouridae
Perlidae
Pteronarcyidae
Hydropsychidae
Philopotamidae
Family Name
June
July
Sept/Oct
Rockbasket


Halfway Brook Macroinvertebrate Family Abundance 2017
Family Abundance
160
140
120
100
80
60
40
20
0
Elmidae
Aeshnidae
Libellulidae
Veliidae
Chironomidae
Tipulidae
Ephemerellidae
Leptophlebiidae
Leuctridae
Perlidae
Brachycentridae
Hydropsychidae
Limnephilidae
Phryganeidae
Family Name
June
July
Sept/Oct
Rockbasket

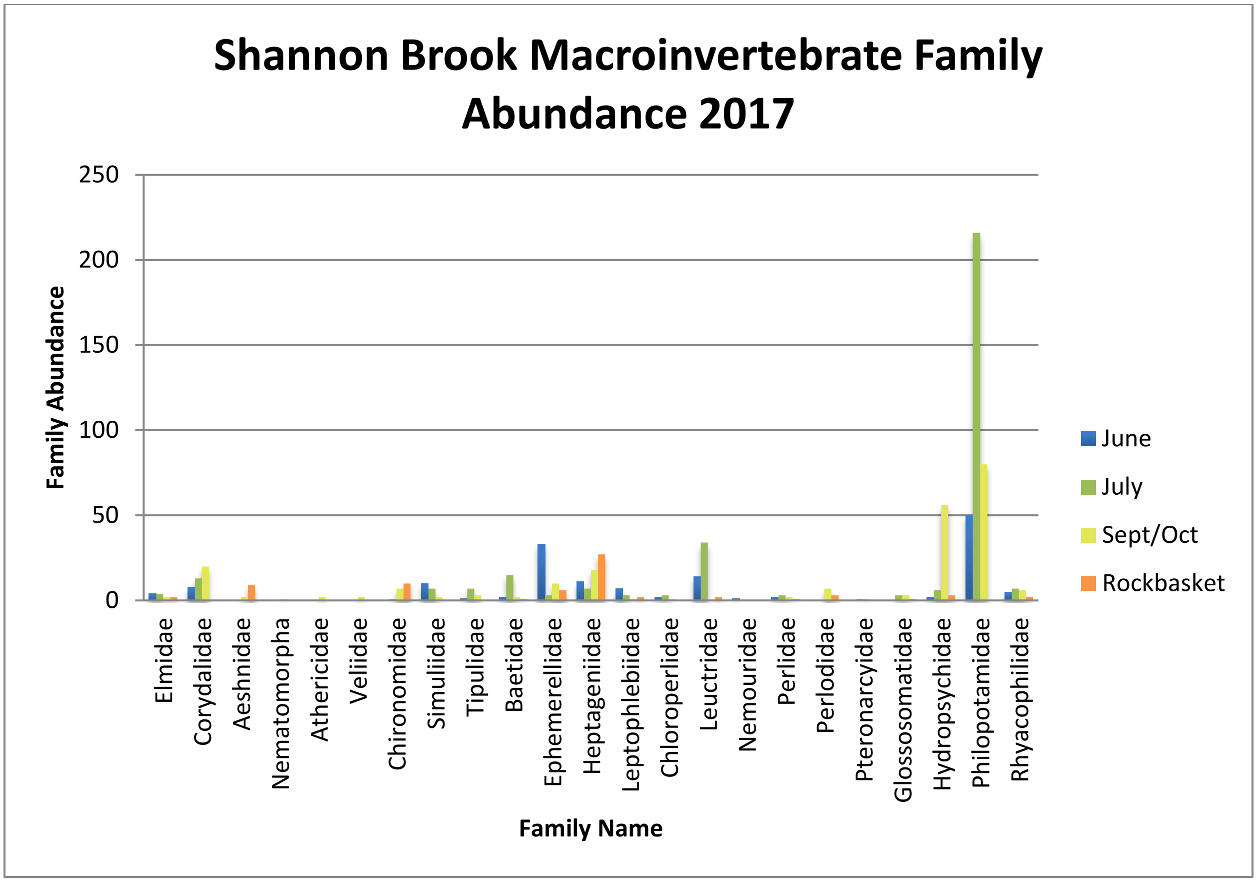

Mad River Macroinvertebrate Family Abundance 2017
Family Abundance
200
180
160
140
120
100
80
60
40
20
0
Elmidae
Gomphidae
Nematomorpha
Athericidae
Chironomidae
Simuliidae
Tipulidae
Empididae
Blephariceridae
Ameletidae
Baetidae
Ephemerellidae
Heptageniidae
Leptophlebiidae
Chloroperlidae
Leuctridae
Nemouridae
Perlodidae
Pteronarcyidae
Brachycentridae
Hydropsychidae
Philopotamidae
Rhyacophilidae
Family Name
June
July
Sept/Oct
Rockbasket



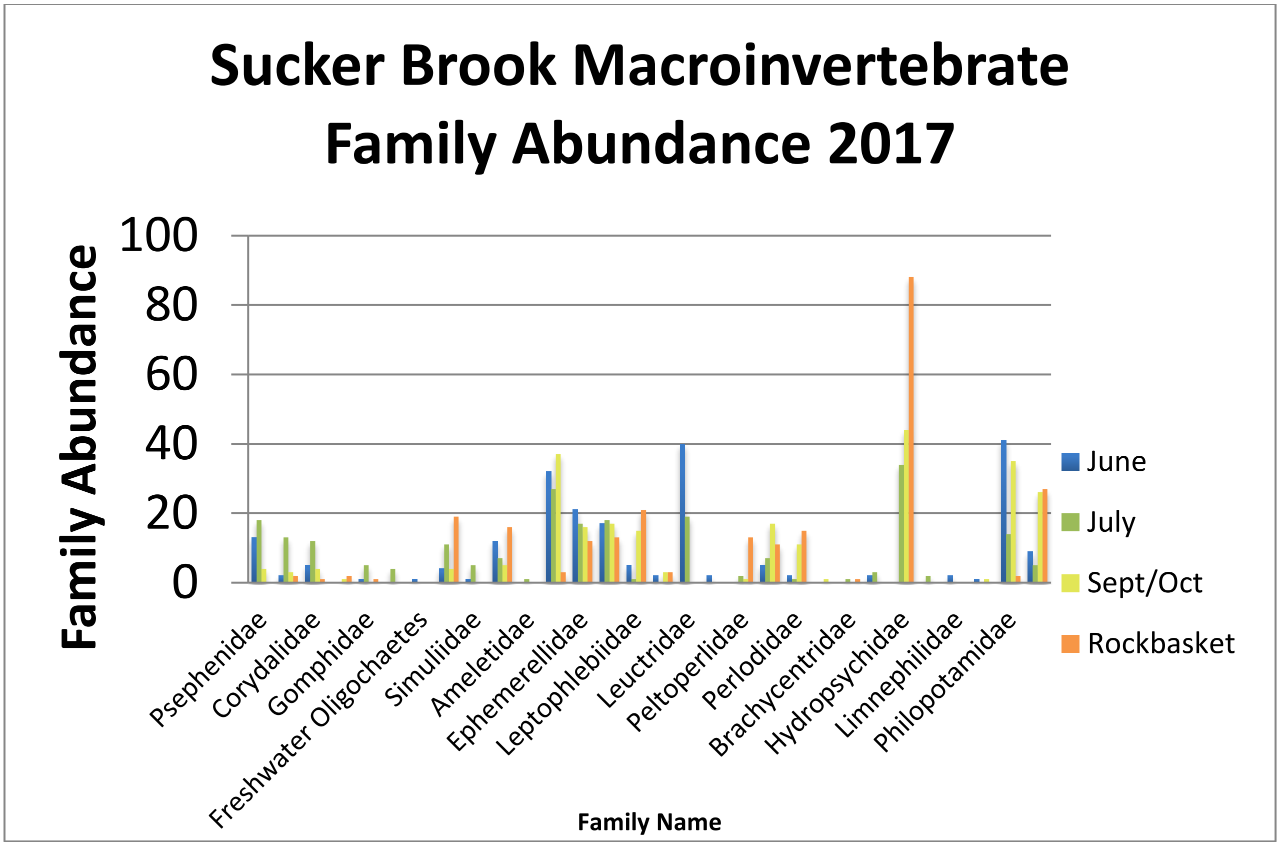
Otter Brook Macroinvertebrate Family Abundance 2017
250
200
150
100
50
0
Family Abundance
Psephenidae
Elmidae
Corydalidae
Aeshnidae
Calopterygidae
Gomphidae
Libellulidae
Athericidae
Veliidae
Chironomidae
Simuliidae
Tipulidae
Ameletidae
Baetidae
Ephemerellidae
Heptageniidae
Isonychiidae
Leptophlebiidae
Chloroperlidae
Leuctridae
Perlidae
Glossosomatidae
Hydropsychidae
Lepidostomatidae
Philopotamidae
Polycentropodidae
Rhyacophilidae
Family Name
June
July
Sept/Oct
Rockbasket

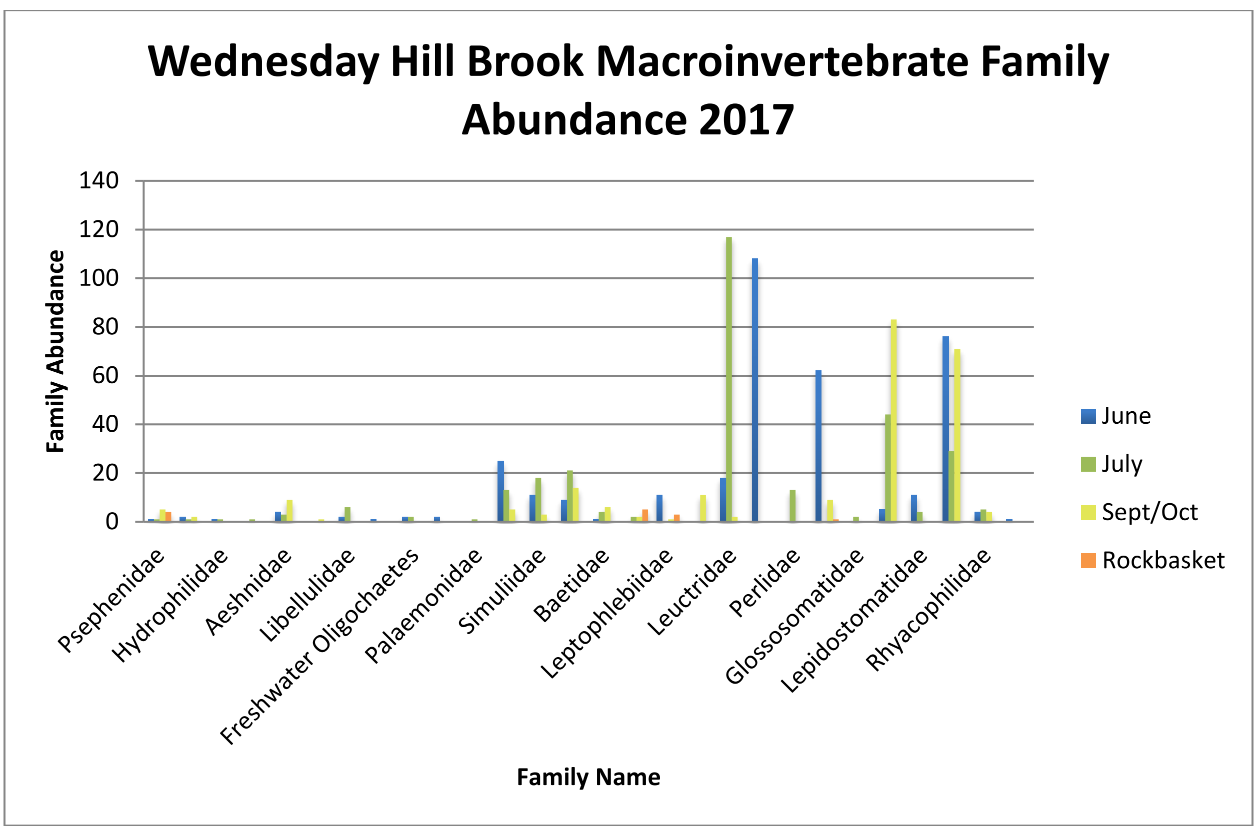


Pemigewasset River Macroinvertebrate Family Abundance 2017
160
140
120
100
80
60
40
20
0
Family Abundance
Elmidae
Corydalidae
Hydracharina
Nematomorpha
Chironomidae
Simuliidae
Baetidae
Ephemerellidae
Leptophlebiidae
Leuctridae
Perlodidae
Brachycentridae
Hydropsychidae
Limnephilidae
Phryganeidae
Rhyacophilidae
Family Name
June
July
Sept/Oct
Rockbasket



Shannon Brook Macroinvertebrate Family Abundance 2017
Family Abundance
250
200
150
100
50
0
Elmidae
Corydalidae
Aeshnidae
Nematomorpha
Athericidae
Veliidae
Chironomidae
Simuliidae
Tipulidae
Baetidae
Ephemerellidae
Heptageniidae
Leptophlebiidae
Chloroperlidae
Leuctridae
Nemouridae
Perlidae
Perlodidae
Pteronarcyidae
Glossosomatidae
Hydropsychidae
Philopotamidae
Rhyacophilidae
Family Name
June
July
Sept/Oct
Rockbasket


Sucker Brook Macroinvertebrate Family Abundance 2017
Family Abundance
100
80
60
40
20
0
Psephenidae
Corydalidae
Gomphidae
Freshwater Oligochaetes
Simuliidae
Ameletidae
Ephemerellidae
Leptophlebiidae
Leuctridae
Peltoperlidae
Perlodidae
Brachycentridae
Hydropsychidae
Limnephilidae
Philopotamidae
June
July
Sept/Oct
Rockbasket
Family Name


Wednesday Hill Brook Macroinvertebrate Family Abundance 2017
Family Abundance
140
120
100
80
60
40
20
0
Psephenidae
Hydrophilidae
Aeshnidae
Libellulidae
Freshwater Oligochaetes
Palaemonidae
Simuliidae
Baetidae
Leptophlebiidae
Leuctridae
Perlidae
Glossosomatidae
Lepidostomatidae
Rhyacophilidae
June
July
Sept/Oct
Rockbasket
Family Name

Appendix B: R-squared values of univariate relationships between chloride and benthic macroinvertebrate metrics from 2017. Values with * are significant (alpha = 0.05), blue reflects a positive and green text a negative relationship with chloride observed.

Response Variable	June-Oct	June	July	Sept-Oct	Rockbaskets
Total Macroinvertebrates	0.0098	0	0.0001	0.0751	0.002
% EPT	0.0043	0.0183	0.0214	0.026	0.003
% Ephemeroptera	0.2863*	0.3406	0.352	0.3053	0.3912
% Plecoptera	0.2206*	0.514*	0.4477*	0.0006	0.2876
% Tricoptera	0.0977	0.1159	0.0132	0.2753	0.0349
% Chironomidae	0.076	0.3276	0.0695	0.0387	0.2161
% Diptera	0.0537	0.1383	0.2483	0.0078	0.362
Total Family Richness	0	0.0154	0.0001	0.1819	0.1514
EPT Family Richness	0.1414*	0.0644	0.3058	0.2209	0.1475
Ephemeroptera Richness	0.2246*	0.2942	0.2292	0.2407	0.0113
Plecoptera Richness	0.2408*	0.1949	0.3903	0.2028	0.4617*
Tricoptera Richness	0.0549	0.0919	0.0676	0.0164	0.0663
Chloroperlidae Relative Abundance ^{IT}	0.0523	0.1968	0.0964	0.0213	0.1827
Leuctridae Relative Abundance ^{IT}	0.0539	0.0005	0.6579*	0.3129	0.039
Philopotamidae Relative Abundance ^{IT}	0.0217	0.1268	0.002	0.1003	0.0076
Rhyacophilidae Relative Abundance ^{IT}	0.0212	0.0362	0.0008	0.1037	0.0433
Simuliidae Relative Abundance ^{T}	0.0018	0.0175	0.0318	0.0023	0.0086

* = significant
positive
negative

Stormwater and Development: How do New Hampshire's communities address the impacts in the land use planning process?

Basic Information

Title:	Stormwater and Development: How do New Hampshire's communities address the impacts in the land use planning process?
Project Number:	2017NH211B
Start Date:	3/1/2017
End Date:	2/28/2018
Funding Source:	104B
Congressional District:	2
Research Category:	Water Quality
Focus Categories:	Management and Planning, Non Point Pollution, Water Quality
Descriptors:	None
Principal Investigators:	June HammondRowan

Publications

There are no publications.

Stormwater and Development: How do New Hampshire's communities address the impacts in the land use planning process?

Annual Report for Project Period March 1, 2017 to February 28, 2017
Project Number: 2017NH211B
PI: June Hammond Rowan

Problem:

Throughout the Unites States, land use in communities is shaped by the decisions made at the local level by Planning Boards or Commissions. Planning Boards have the responsibility to create land use plans and regulations, implement local regulations, and make decisions about applications for development which often result changes in land use and urbanization. When development occurs, there typically is an increase in impervious surfaces which alters stormwater runoff patterns and results in changes to both the hydrology and water quality (EPA, 2016).

Stormwater has long been recognized to be a major cause of nonpoint source pollution and is one of the leading causes of water pollution nationally (DES, 2016). Since the 1980s, efforts have been made to address the impacts of stormwater from development. Government agencies and non-profit organizations provide endless resources aimed at improving how runoff is managed. Much of this effort has been directed at planners and Planning Boards to change the way stormwater is managed at the site scale. Traditionally, stormwater has been managed by moving it off site through engineered systems. Over the past 25 years, techniques such as low impact development, best management practices, smart growth, new urbanism, conservation planning, and green infrastructure have emerged to help treat stormwater on site and control both the quantity and quality of runoff from new development. It is now recognized that stormwater also needs to be managed regionally, typically at the watershed scale.

Municipalities have had years to both recognize the impacts of development on water quantity and quality and find ways to mitigate these impacts. Although efforts have been made to improve watershed planning, in New Hampshire local Planning Boards are our land use decision makers. They make both land use plans and regulations as well as approve applications for development. Stormwater impacts from development are therefore managed locally through the planning process. This project provides a statewide assessment of the local land use plans, policies, and regulations that address the impacts from development on water resources.

Objectives:

The goal of the project is to assess how stormwater is addressed in both Master Plans and land use regulations across New Hampshire. The project objectives are to:

1. Determine what and how New Hampshire municipalities are doing to address stormwater impacts from development in their land use plans and regulations;

2. Assess if and how stormwater management goals are implemented in land use regulations and reveal potential disconnects between plan goals and implementation strategies in protecting water quality and quantity;
3. Identify both commonalities and differences in the ways municipalities are addressing stormwater impacts across New Hampshire to help identify best practices;
4. Share research findings with organizations to enhance state and regional stormwater management efforts; and
5. Provide a Plymouth State University graduate student an opportunity to assist with this research and learn about land use planning.

<u>Methods</u>:

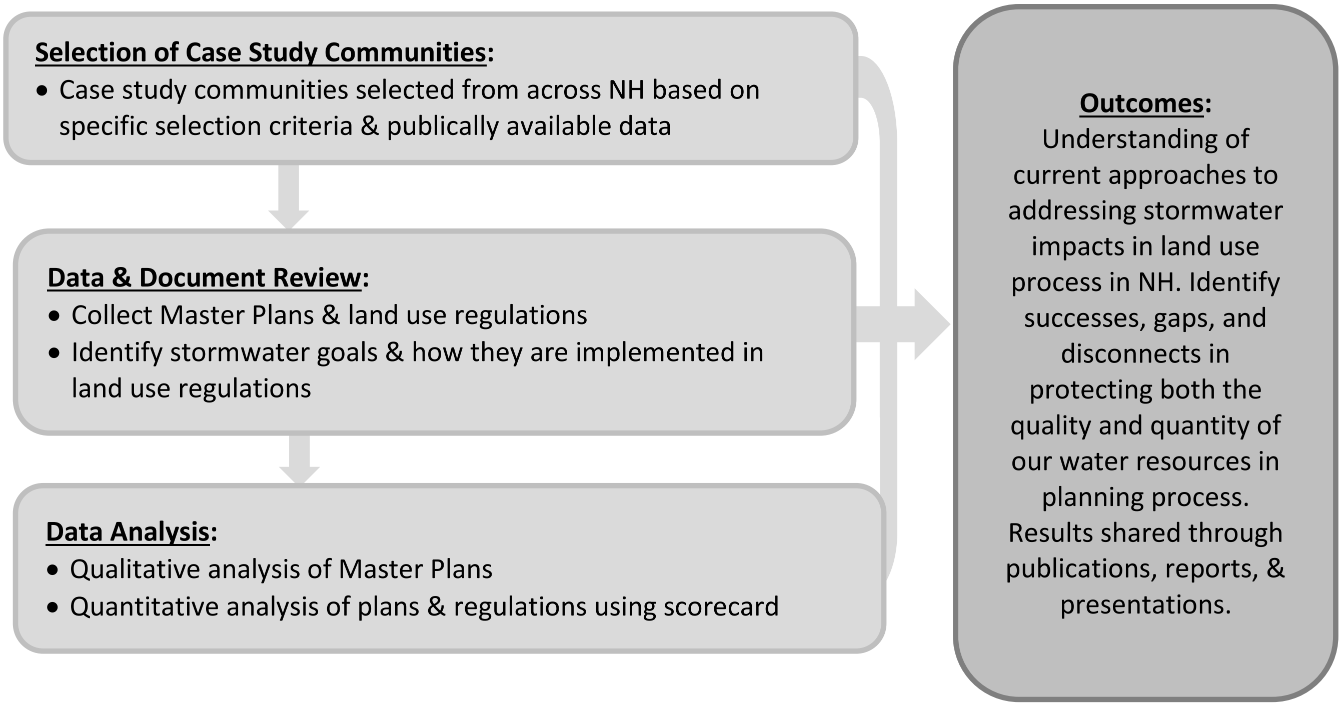
The geographic scope of our research includes case study communities across the State of New Hampshire. A case study research approach is typical in land use planning research. It is used to answer questions involving "how" and "why" leading to insight about our communities while also providing generalizable results (Yin, 1994). 57 municipalities were selected distributed across New Hampshire's nine Regional Planning Commissions. Case study communities were purposely selected in order to gain adequate data and representation across the state. Sample selection criteria included location, size, planning staff in the community, and the quality and availability of data sources.

We collected Master Plans, Subdivision Regulations, Site Plan Review Regulations, and Zoning Ordinances from the case study communities. These documents serve as our data for the study and analysis involves a mixed method approach consisting of:

1. Qualitative analysis: Master Plans and land use regulations are being analyzed to determine the content, themes, similarities and differences across communities regarding stormwater management goals and objectives using NVivo qualitative research software.
2. Quantitative analysis: Each case study community's Master Plan and land use regulations will be scored using a scorecard system adapted from existing stormwater assessment scorecards. Scores will provide a quantitative measure to assess the means and ways communities are addressing stormwater impacts.

Our qualitative analysis allows for a deeper understanding of stormwater management goals across New Hampshire and our quantitative analysis will provide an assessment of what and how our municipalities are doing across the state to manage stormwater in local plans and regulations. The goal is not to compare one municipality to another, but rather to determine the success of the state in implementing stormwater management techniques in the land development process. This approach will highlight communities that may have a disconnect between stormwater goals in their Master Plan and the implementation of these goals in land use regulations. The research methodology is summarized in Figure 1.

Figure 1. Conceptual design of research.



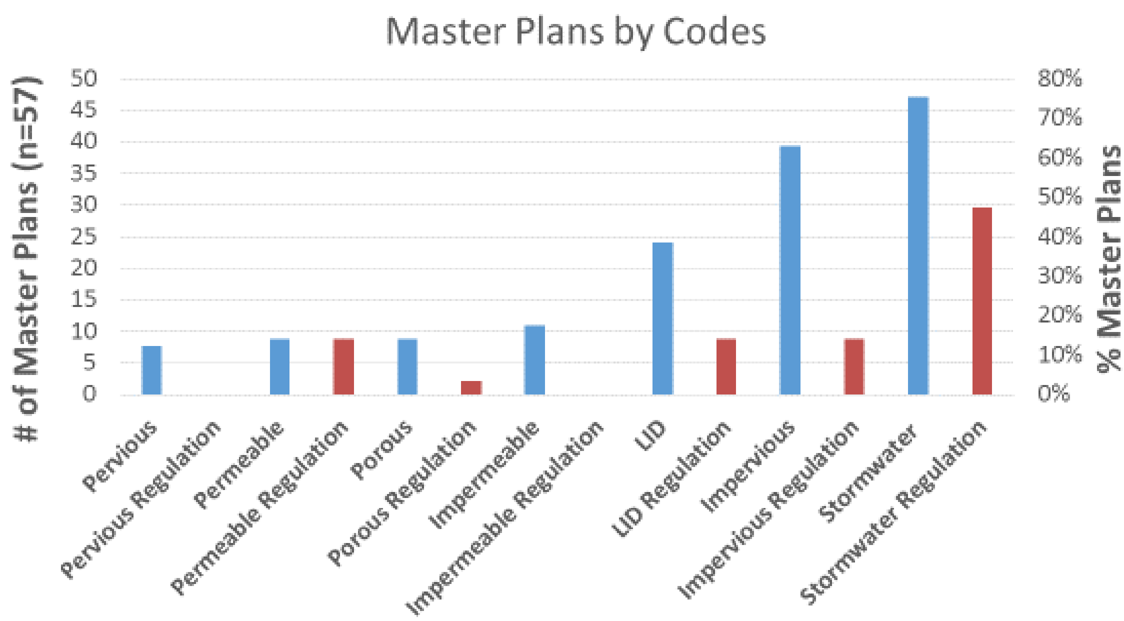
Initial Findings and Significance:

Funds for the project were delayed and not received until August 2017. However, in the spring of 2017, with matching support from Plymouth State University, the selection of case study communities, data collection (Master Plans and land use regulations), and analysis and coding of stormwater related terms in Master Plans was completed. Two graduate students worked on the project in the fall of 2017 focusing on reviewing subdivision and site plan review regulations from case study communities for design standards related to stormwater management. In the winter of 2018, one of the graduate students continued this work focusing on zoning ordinances and how these regulations address stormwater. Analysis is ongoing.

Findings to date indicate that Master Plans do mention stormwater and related terms (see Figure 2.), but, most often, these references are in the context of background information about the topic. Statements about stormwater are vague and general in nature. Master Plans provide limited community based guidance related to stormwater management. Except for suggesting a need for limits to impervious surface, the plans provide few specifics for land use regulations.

The project is continuing analysis of land use regulations and assessing the ways and means communities address stormwater impacts.

Figure 2. Occurrence of Stormwater Related Terms in New Hampshire Master Plans.



<u>Publications and presentations</u>:

Hammond Rowan, June; Jason Spencer and Carolyn Greenough. “How do New Hampshire Communities Address Stormwater in their Master Plans?” Presentation at New Hampshire Water & Watershed Conference, March 23, 2018.

<u>Notable awards and achievements</u>:

Carolyn Greenough, a graduate student who assisted with the research in spring of 2017, completed her MS in Environmental Science and Policy in May 2017. Jason Spencer, a graduate student supported by this project, will complete his MS degree in Environmental Science and Policy in May 2018.

<u>Publications from WRRC supported work completed in previous years and not reported previously</u>:

Not applicable.

<u>Outreach or Information Transferred</u>:

Preliminary findings of this research were presented at the 2018 New Hampshire Water and Watershed Conference on March 28, 2018. This conference is a key event for sharing current water resource information and, in 2018, focused on regional environmental stresses and how we are adapting to new information, emerging issues, and current events affecting water quality and water supply. The event drew 160 attendees from watershed associations, conservation organizations, municipal staff from public works and planning departments, local volunteer Planning Board and Conservation Commission members, representatives from state and federal agencies, environmental consultants, researchers, educators, and students.

Number of Students Supported:

This project has provided partial direct support for two graduate students in Plymouth State University's MS in Environmental Science and Policy program (Jason Spencer and Nichole Stevens) through support for tuition and a stipend. An additional MS graduate student (Carolyn Greenough) was supported through project matching funds from Plymouth State University.

Faculty Supported:

PI June Hammond Rowan, Research Assistant Professor, was supported by Plymouth State University as financial match to the project.

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US Environmental Protection Agency (EPA). (2016) Problems with Stormwater Pollution. https://www.epa.gov/npdes/npdes-stormwater-program

Yin, R.K. (1994). *Case study research* (2nd ed.). Thousand Oaks: Sage Publications.

Information Transfer Program Introduction

The NH WRRC supported one information transfer project:

1. New Hampshire WRRC Information Transfer

New Hampshire WRRC Information Transfer

Basic Information

Title:	New Hampshire WRRC Information Transfer
Project Number:	2008NH97B
Start Date:	3/1/2008
End Date:	2/28/2018
Funding Source:	104B
Congressional District:	01
Research Category:	Not Applicable
Focus Categories:	Management and Planning, Education, Non Point Pollution
Descriptors:	None
Principal Investigators:	William H. McDowell, Michelle Daley Shattuck

Publications

1. Baillio, J. 2012. 2012. Controls on variability of dissolved greenhouse gas concentration and emissions from small streams in southeastern New Hampshire. M.S. Dissertation, Department of Natural Resources & the Environment, College of Life Science and Agriculture, University of New Hampshire, Durham, NH, 111 pages.
2. Daley, M.L. and W.H. McDowell, In Preparation, Human impacts on stream nitrogen chemistry and watershed N retention across a wide range of rural to urban catchments, Ecological Applications.
3. Hope, A.J., W.H. McDowell, W.M. Wollheim, Submitted, Ecosystem metabolism and nutrient uptake in an urban, piped headwater stream, Biogeochemistry.
4. Liptzin, D., M.L. Daley, and W.H. McDowell. Accepted. A comparison of wet deposition collectors at a coastal rural site. Submitted to Water, Air, & Soil Pollution. April 2013.
5. Parham, L. 2012. Spatial and temporal variation in degradation of dissolved organic carbon on the main stem of the Lamprey River. M.S. Dissertation, Department of Natural Resources & the Environment, College of Life Science and Agriculture, University of New Hampshire, Durham, NH, 66 pages.
6. Hope, A.J., W.H. McDowell, W.M. Wollheim. 2013. Ecosystem metabolism and nutrient uptake in an urban, piped headwater stream. Biogeochemistry. September 2013. DOI 10.1007/s10533-013-9900-y
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Information Transfer

Unbridled development and population growth can have detrimental impacts to water resources and ecosystem services. Rapid population growth is occurring in New Hampshire and state regulations, planning board decisions and zoning classifications all attempt to minimize the environmental impact of this rapid population growth. Most land use planning decisions are made at the local level on a town by town basis, often by volunteers who serve on various boards, commissions and committees. Decisions by these various resource managers are often made without a full understanding of the consequences that their decisions will have on water resources or ecosystem services.

This project provided salary for the Center's Director and Associate Director to meet with state representatives, local town officials, watershed groups, school groups, the general public and scientists to discuss WRRC findings that relate to population growth, land use change and climate variability. Over the past year, the NH WRRC meet with the following organizations to discuss water resource issues: Durham Unitarian Universalist Fellowship, NH Fish and Game, Natural Resources Conservation Service (NRCS), Trout Unlimited (TU), Piscataqua Region Estuaries Partnership (PREP), NH Department of Environmental Services (DES), the US Geological Survey and the US Environmental Protection Agency (EPA). The NH WRRC website (http://www.wrrc.unh.edu/) is also used to disseminate information on water resources, and is updated and maintained by salary provided by this project. The Director and Associate Director dedicate time discussing current and future research in the Lamprey River Hydrologic Observatory, which is partially funded by the longstanding 104B project "Water Quality and the Landscape: Long-term monitoring of a rapidly developing suburban watershed". On January 8, 2018 the NH WRRC funded and organized the **Eleventh Annual Lamprey River Symposium** (see also below). Presentations focused on nutrients and other solutes, bacteria, sediment, hydrology, groundwater, climate and land use change, water quality indicators and monitoring programs in coastal New Hampshire. The symposium attracted approximately 90 attendees, including scientists, regional leaders, town officials, members of state agencies, and federal agencies. The agenda can be found on the NH WRRC Lamprey River Hydrologic Observatory Symposium website. This annual symposium and other discussions in which the Center's Director and Associate Director participate further the research and information transfer goals of the NH WRRC.

2017 Information Transfer Activities Supported by Section 104b Funding and Matching Funds

Data sharing with Lamprey River watershed local advisory committee

The Lamprey River Advisory Committee (LRAC) is undergoing a long-term analysis of Lamprey River water quality data collected by both the Lamprey River Watershed Association's (LRWA) volunteer monitoring program and the NH WRRC 104B project "Water Quality and the Landscape: Long-term monitoring of a rapidly developing suburban watershed". The NH WRRC associate director serves on the LRAC and is a member of the water quality sub-committee which is advising a LRAC funded intern who is conducting the long-term water quality analysis. Temporal and spatial trends in dissolved oxygen, pH and nitrate have been examined thus far and further analysis is underway.

Nitrogen Data in New Hampshire's Great Bay watershed

Over the last nine years, there has been significant focus on nitrogen loading to New Hampshire's largest estuary, the Great Bay estuary, and the impairment to aquatic life it has caused. In August 2009, Great Bay, Little Bay and the tidal rivers were added to the New Hampshire 2008 303d list of impaired waters rendering them in violation of the federal Clean Water Act. Based on the most recent "State of Our Estuaries Report" prepared by PREP (2018), 33% of the nitrogen entering Great Bay and Little Bay is from point sources; the majority (67%) enters via non-point sources of pollution. The Lamprey River is the largest tributary to Great Bay, and thus the long-term data provided by the NH WRRC from the LRHO are of considerable value for watershed management. The NH WRRC provides the best dataset in NH for assessing the spatial and temporal variability in N concentrations and export in response to suburbanization and changes in land use. These 17+ years of data will be instrumental in assessing the success of current and future efforts to reduce non-point sources of nitrogen pollution reaching Great Bay. There is much interest in LRHO datasets from NH DES, PREP, EPA and other municipal, regional, state and federal agents. Many of the presentations listed below and meetings attended focused on transferring information on nutrient cycling to stakeholders throughout NH's coastal watershed and beyond. The NH WRRC has received several phone calls and meeting requests to discuss the Great Bay nitrogen issue. The NH WRRC was also asked by PREP to update the nutrient loading indicator for the 2018 State of Our Estuaries report.

Water quality monitoring advice for wood restoration projects in NH streams

The Natural Resources Conservation Service and TU have selected 23 Wetlands Reserve Program (WRP) properties in NH for wood loading restoration work. The project involves adding wood into small segments of 1st and 2nd order stream channels (averaging about 1,000 feet) with a primary goal of recreating and increasing fish spawning and rearing habitat as well as preventing bank erosion and improving stream geomorphology. A supplemental goal of this work is to study the changes in water quality and nutrient uptake which may be enhanced by adding carbon (in the form of wood) to streams. The NH WRRC Director, Associate Director and the WQAL manager have been advising the NRCS and TU on how to best understand changes in water quality and nutrient dynamics with existing financial resources. With collaboration between the NRCS, TU and the NH WRRC, baseline water quality monitoring began in 2014. Wood installations occurred mainly in 2015 and 2016 and a few properties were restored in 2017.

Drinking water quality in New Hampshire

The recent Perfluorooctanoic Acid (PFOA) and lead contamination of southern NH drinking water has prompted several inquiries to the NH WRRC and the Water Quality Analysis Laboratory (WQAL) from residents and local media concerned with drinking water quality in the state.

Symposia, Conferences and Seminars Organized and Funded

The NH WRRC funded and organized the "**Eleventh Annual Lamprey River Symposium**" held January 8, 2018 in Durham, NH. The symposium is dedicated to exchanging the results of recent research on the water quality, hydrology, water resources issues, and management of the Lamprey River basin. The Symposium is a vehicle for researchers to share data and insights with other researchers, as well as those in the management and policy arena who would benefit from exposure to the latest research on the watershed. The symposium drew approximately 90 attendees, including researchers, legislators, water system operators, town officials, regional leaders and government officials. The symposium contained 8 presentations split up over three sessions with ample time for discussion. The day ended with an open discussion on research priorities in the Lamprey watershed and southeast NH. This event was funded and organized by the NH WRRC. NH EPSCoR assisted with registration and printing. Survey results indicate that most of the attendees found the topics covered to be either helpful or very helpful.

The NH WRRC sponsored the 10th annual "**NH Water and Watershed Conference**" which was held on March 24, 2017 in Plymouth, NH. This event was designed to meet the information and networking needs of lake, river, and watershed groups; environmental organizations; volunteer monitors; municipal board and staff members; elected officials; local and regional planners; policy makers; scientists; educators; consultants and students. The 2017 conference was focused on what has transpired during the past ten years and what we might expect during the next ten years and beyond. The NH WRRC co-sponsored this conference along with Plymouth State University and the Center for the Environment, NH EPSCoR, NH DES, US Geological Survey New England Water Science Center and a few others. The conference featured a plenary speaker, approximately 30 talks, a poster session, and a workshop style session in the afternoon designed to summarize progress over the past 10 years. The conference drew approximately 250 people, including researchers, legislators, water system operators, land use planners, and government officials. The Center's Associate Director also serves on the planning committee for the annual NH Water and Watershed Conference.

Information transfer activities of the NH WRRC

Publications

Bucci, J. P., M. D. Shattuck, S. A. Aytur, R. Carey and W. H. McDowell (2017). A case study characterizing animal fecal sources in surface water using a mitochondrial DNA marker. Environmental Monitoring and Assessment 189(8).

Contosta, A. R., A. Adolph, D. Burchsted, E. Burakowski, M. Green, D. Guerra, M. Albert, J. Dibb, M. Martin, W. H. McDowell, M. Routhier, C. Wake, R. Whitaker and W. Wollheim (2017). A longer vernal window: the role of winter coldness and snowpack in driving spring transitions and lags. Global Change Biology 23(4): 1610-1625.

Hunt, C. W., L. Snyder, J. E. Salisbury, D. Vandemark and W. H. McDowell (2017). SIPCO2: A simple, inexpensive surface water pCO(2) sensor. Limnology and Oceanography-Methods 15(3): 291-301.

Koenig, L.E., Shattuck, M.D., Snyder, L.E., Potter, J.D. and McDowell, W.H. 2017. Deconstructing the effects of flow on DOC, nitrate, and major ion interactions using a high-frequency aquatic sensor network. Water Resources Research. 53: 10,655–10,673. DOI: 10.1002/2017WR020739.

Snyder, L.E., Potter, J.D. and McDowell, W.H. 2018. An Evaluation of Nitrate, fDOM, and Turbidity Sensors in New Hampshire Streams. Water Resources Research. Special issue “Continuous nutrient sensing in research and management: applications and lessons learned across aquatic environments and watersheds”. DOI: 10.1002/2017WR020678

Wymore, AS, J Potter, L Snyder, B Rodríguez-Cardona, and WH McDowell. 2018. Using in-situ optical sensors to understand the biogeochemistry of dissolved organic matter across a stream network. Water Resources Research. DOI: 10.1002/2017WR022168.

Conference Proceedings & Abstracts:

Contosta, A., Burchsted, D., Burakowski, E., Green, M., Guerra, D., Albert, M., Dibb, J., Martin, M., McDowell, W.H., Routhier, M., Wake, C., Whitaker, R. and Wollheim, W. 2017. A longer vernal window: How winter coldness and snowpack influence spring transitions and lags. Northeast Ecosystem Research Cooperative Conference. March 28-29, 2017, Saratoga Springs, New York, USA.

Goodale, C. N. Ohte and WH McDowell. 2017. Convened the Biogeochemistry of nitrogen session at the 9th International Symposium on Ecosystem Behavior. BIOGEOMON August 20-24, 2017. Litomyšl Chateau, Czech Republic.

Koenig, L.E., L.E. Snyder, A.P. Appling, C. Hunt, J.D. Potter, W.H. McDowell. 2017. Annual patterns in aquatic metabolism and CO2 emissions from New Hampshire streams (Oral). June 4-9, 2017 Society for Freshwater Science meeting, Raleigh, NC.

McDowell, W.H., J. Potter. 2017. Tradeoffs in Greenhouse Gas Fluxes from Aquatic Ecosystems Along a Rural to Urban Gradient are Driven by N Loading. 2017 Fall Meeting, AGU, New Orleans, LA, 11-15 Dec.

Shattuck, M.D. and McDowell, W.H. 2017. U.S. Geological Survey - New England Water Science Center Science Symposium: Coastal Nitrogen Research. April, 18, 2017. Worcester, MA.

Shattuck, M.D., Koenig, L. Potter, J.D., Snyder, L.E. and McDowell, W.H. 2017. Regional coherence in solute interactions during stormflow in a statewide aquatic sensor network. NH Water & Watershed Conference. March, 24, 2017. Plymouth, NH.

Wymore, A., S. Kaushal, W.H. McDowell, P. Kortelainen, E. Bernhardt, P. Johnes, W. Dodds, S. Johnson, J. Brookshire, R. Spencer, B.Rodriguez-Cardona, A. Helton, R. Barnes, A. Algerich, S. Haq, P. Sullivan, C. Lopez-Lloreda, A. Coble, M.D. Shattuck. 2017. Carbon and nitrogen stoichiometry across stream ecosystems. 2017 Fall Meeting, AGU, New Orleans, LA, 11-15 Dec.

Presentations/Information Transfer

Koenig, L. 2017. For the 5th consecutive year, Koenig served as the instructor for the STEM mini-course offered August 21st-25th, 2017 through the CONNECT program at UNH (http://www.unh.edu/connect/). The objective of the course is to provide an opportunity for incoming freshmen that come from groups with historically low retention in STEM majors (e.g. low-income, multicultural, first-generation college students) to build community, discover college resources, and bolster skills that are needed to succeed in their academic programs (e.g. writing of lab/research reports, basic math and statistics for analyzing scientific data). There were 13 students in the class, but the broader CONNECT program served approximately 80 students in 2017.

McDowell, W.H. 2017. Taking river biogeochemistry into the fourth dimension. Presentation to NSF EPSCoR program officers. 20 April, 2017.

McDowell, W.H. 2017. The NH Agricultural Experiment Station at the University of New Hampshire welcomed Dr. Catalino Blanche, national program leader in the Division of Environmental Systems with the U.S. Department of Agriculture. Dr. Blanche provides national leadership for forestry and research programs related to the production, protection, and utilization of forest resources, including for the McIntire-Stennis Cooperative Forestry Program. Dr. McDowell discussed with Dr. Blanche the nitrogen issues in Great Bay and the role of nitrogen cycling in suburbanizing forested watersheds that drain to the bay. Dr. McDowell also discussed the NH EPSCoR Ecosystems & Society project's investment in scientific instrumentation to build a sensor network and showed Dr. Blanche the aquatic sensors installed in the Lamprey River (the largest tributary to Great Bay) at Wiswall Dam in Durham, NH. April 26, 2017.

McDowell, W.H. and Shattuck, M. D. "Nonpoint Nitrogen Sources and Transport in New Hampshire's Great Bay Watershed". Presented 31 October 2017, at the workshop entitled "Nitrogen, Septic Systems, Great Bay and Why it Matters", Great Bay National Estuarine Research Reserve, Greenland, NH.

Shattuck, M.D. and McDowell, W.H. 2017 Nonpoint nitrogen sources and transport in the Great Bay watershed. Unitarian Universalist Church. April 30, 2017. Durham, NH.

USGS Summer Intern Program

None.

Student Support					
Category	**Section 104 Base Grant**	**Section 104 NCGP Award**	**NIWR-USGS Internship**	**Supplemental Awards**	**Total**
Undergraduate	17	1	0	4	22
Masters	9	1	0	1	11
Ph.D.	3	0	0	1	4
Post-Doc.	3	0	0	1	4
Total	32	2	0	7	41

Notable Awards and Achievements

Director Dr. William H. McDowell received the 2017 Distinguished Professor Award. The purpose of this award is to identify and honor longstanding members of the University of New Hampshire faculty. This singular university-wide award will be given each year to the faculty member whose overall record of excellent teaching, caring about students, devotion to the university community and substantial record of scholarly achievement exemplifies what we would call a ‘distinguished career’.

Ursula Jongebloed served as an USGS intern on the project “Investigations into the bioavailability and bioaccumulation of selenium (Se) and mercury (Hg) in the San Francisco Bay Estuary”. Ursula began the internship at the USGS National Research Program office in Menlo Park, California in June 2015 after completing her sophomore year at Dartmouth College (located in Hanover, New Hampshire). The internship was completed in May 2016 and the experience was very rewarding for both Ursula and her USGS mentor Robin Stewart. The internship experience was so successful that Ursula is preparing a manuscript along with co-authors Robin Stewart and Amy Kleckner on the trends in dissolved and particulate selenium concentrations with respect to bivalve Se concentrations and water year in the San Francisco Estuary.

Rodriguez-Cardona, B. (PhD student supported by project 2003NH21B) was awarded a 2017 Chateaubriand Fellowship. The Chateaubriand Fellowship is a grant offered by the Embassy of France in the United States. It supports outstanding Ph.D. students from American universities who wish to conduct research in France for a period ranging from 4 to 9 months. Chateaubriand fellows are selected through a merit-based competition, through a collaborative process involving expert evaluators in both countries. Bianca has travelled the globe, studying carbon and nitrogen coupling in streams across biomes. She traveled to Paris, France for this fellowship.

Currently NH has numerous watersheds listed as impaired due to elevated chloride levels resulting from salt use in winter road maintenance with most those watersheds located in the southern part of the state. College Brook is one of the impaired watersheds and the impairment listing was based on data produced from the 2003NH21B project.

Co-PI Celia Chen and investigator Vivien Taylor (2016NH205G) received a CompX grant through the Neukom Institute at Dartmouth to compare findings from Lake Sunapee with other watersheds. The CompX grant has enabled us to improve our GIS and modeling capabilities.

Investigator Vivien Taylor (2016NH205G) received an NSF grant to study sources of methylmercury to fish in Lake Sunapee. This work will build on the current grant by comparing the isotopic ratios of mercury in the tributaries and in the lake, to determine whether bioavailable mercury is transferred from the watershed to the lake, or produced from in-lake processes.

Keith Kantack joined the WRRC 2016NH205G research team while finishing up his Master’s degree in Earth Science, and applied his expertise in remote sensing and mapping to analyzing watershed landscape characteristics of Lake Sunapee and three reference watersheds. Keith also expanded his research skills by collecting and processing stream water samples for our field study, and learned to identify and collect biofilm and invertebrate samples for ecological assessment. Following the field season, he led a field laboratory session on stream invertebrates as part of the Dartmouth ‘Stretch’, an off-campus field program for Earth Science majors. Since gaining applied research experience on the WRRC Lake Sunapee project, Keith has started working for a river restoration company in New England, and is advancing his career in watershed research. Keith Kantack (Dartmouth MSc Earth Science 2017) commented that “Being a part of the Dartmouth WRRC team was a great opportunity to apply some of my GIS and watershed science skills, but also to learn hands on about aquatic ecology and environmental chemistry. My experience on the team made me a stronger applicant for the job I now have with a river restoration firm, where I work to improve riparian habitat in the New England and the Pacific Northwest."

Dr. Amy Villamagna (2016NH201B and 2017NH210B) was honored with the Helen Abbott Endowed Professors of Environmental Studies (2016-2020) for her research on the environment and engagement of students in research.

Katerina Crowley (2017NH210B) was awarded the Marapesse Scholarship in 2017 for her participation in research on Sucker Brook, a tributary to Webster Lake (NH). Katerina was also awarded second place in a student poster competition at the 2018 New England Association of Environmental Biologists in Devens, MA.